Introduction to
CHEMICAL PHARMACOLOGY

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R. B. Barlow

Senior Lecturer in Pharmacology, University of Edinburgh

WITH A FOREWORD BY

H. R. Ing, F.R.S.

Reader in Chemical Pharmacology, University of Oxford

SECOND EDITION

Foreword

THE subject of this book – namely, the structure action relationships of drugs or, to use an old fashioned phrase, the relation between chemical constitution and physiological action – is one that has fascinated chemists, particularly organic chemists, for many years It has been a somewhat disappointing subject of study, and some workers have despaired of finding any convincing relationships

A J Clark, in his classical monograph on *General Pharmacology*, concluded that there were 'scarcely any general rules discernible and that every cell drug system was a law unto itself' And yet the chemist has an underlying conviction that there must be some chemical explanation of the way in which drugs modify the functions of living organisms, that the fundamental problem of how drugs act is a chemical problem Of course the chemist is right, we can only think of the drug itself in chemical terms, and, consequently, theories of drug action must also be expressed in chemical terms A possible viewpoint (it can scarcely rank as a theory) is that drugs modify the functions of living organisms by interfering in some way with the biochemical reactions which are continuously going on in living cells The difficulty is that the chemist knows so little about the intimate chemical nature and properties of the physiological structures upon which drugs exert their disturbing effects No doubt the biochemist will ultimately be able to tell him about these things, but meanwhile the chemist ought to pay more attention than he has usually done to what the pharmacologist has to say One of the most important functions of the pharmacologist is to disentangle the physiological mechanisms by which a given drug action is achieved Superficially similar physiological effects can be produced by a variety of mechanisms, the chemist is frequently apt to forget this, he is often unaware of the fact that changes in structure, sometimes quite small changes, may alter not only the intensity of drug action but also the mechanism by which some particular physiological result is achieved The whole problem of the structure action relationships of drugs involves two separate inquiries how changes in structure affect the mechanism of action and how they influence the intensity of action, when the mechanism is unchanged The chemist is apt to think only about how changes in structure affect the intensity of some physiological response No wonder he is frequently puzzled by the astonishing changes in intensity which small alterations of structure produce Even if the pharmacologist can provide evidence that a group of related drugs are all acting in the same way, the problem of relating intensity of action to structure is difficult enough, but if changes of structure alter the mechanism of action, the chemist may easily be led astray

Dr Barlow's books is an attempt – almost the first attempt – to instruct chemists about these matters Dr Barlow is not only an organic chemist, he

has practical experience of testing the pharmacological activity of compounds which he has made, he can, therefore, speak with authority to chemists about these matters, and it is my hope that his book will encourage chemists, particularly young chemists, to enter this curious and fascinating borderland between chemistry and biology, and to learn more than chemists have usually been willing to learn about the biological side of the subject

What is obviously needed is a much closer liaison between the chemist and the pharmacologist, but if this liaison is to be achieved, the chemist must try to understand the nature of the problems which face the pharmacologist, and the pharmacologist must try to understand how the chemist thinks about the compounds that he makes There have been in the history of pharmacology some famous combinations of chemist and pharmacologist – e g , Crum Brown and Fraser, Reid Hunt and Renshaw, Barger and Dale, &c. These names alone are key names in the development of pharmacology Pharmacology, which has remained for so many years a rather inferior relation of physiology, can only achieve its proper status as an independent branch of medical science, if it is willing to invite the co-operation of the chemist, but the chemist, fascinated as he usually is by synthetical problems, must, if he is interested in the structure-action relationship of drugs, he prepared to learn something about pharmacology, about the curious ways in which drugs modify the functions of living organisms The whole history of the study of the structure-action relationships of drugs is befogged by the circumstance that the pharmacologist did not understand what the chemist had in his mind and the chemist did not understand what the pharmacologist was doing In my opinion the chemist ought to watch the pharmacologist at work on the testing of new compounds, only so can he appreciate the fascination of pharmacology, and understand the curious paradox that although the pharmacologist can detect, and even estimate, amounts of chemical substances well below those which can be detected or estimated by chemical means, he can only get approximate measures of activity, because of the individual variation of living organisms to drugs

My own belief is that the chemist and the pharmacologist, if they are prepared to understand each other's point of view, can jointly, but not independently, make important advances in a subject which is vital to therapeutics How drugs act is, as I have said, a chemical problem, but the chemist alone cannot solve it, he can solve it only if he is prepared to work hand in glove with the pharmacologist, so that Dr Barlow's book, which attempts to instruct chemists about these difficult problems of drug action, receives my warm recommendation to the reader

H R ING

Department of Pharmacology,
Oxford

Author's Note to the Second Edition

This book has been almost completely re-written The decision to omit any detailed consideration of the actions of drugs on the central nervous system was taken in order to limit the size of the book and the time which its revision was demanding It was also felt that, with a few exceptions, there was not really enough known about the mode of action of drugs on the central nervous system to justify their inclusion in an introduction to chemical pharmacology

The Appendix, however, has been retained because it is thought that this is of real value to chemists who cannot be expected at this stage to look up more detailed works It is for the biologist to explain the subject in simple terms to the chemist so that the chemist may see where his chemical experience may be relevant Although the chemist should be aware of his ignorance, it should not be necessary for him to undergo a full formal training in biology before his comments are attended to, otherwise there is the serious danger that a subject such as chemical pharmacology will be explained in terms of the chemistry of yesterday rather than that of today I appreciate that this criticism may be levelled at sections of this book and would be glad to be informed of errors or criticisms of any sort

Names of Drugs Compounds are referred to by their chemical names when these are simple, failing this, by their pharmacopoeial names (indicated by an initial capital letter) and failing this by their approved names or a trade name (indicated by italics)

Optical Isomers The signs (+) and (−) refer to optical rotation Where it is possible to assign an absolute configuration I have used the signs R- and S-, following the convention of Cahn, Ingold, and Prelog (*Experientia*, 1956, **12**, 81) This avoids many ambiguities, (−) adrenaline, for instance, has the L-configuration with reference to glyceraldehyde but the D- configuration with reference to serine whereas the description R- is unambiguous Even the substance, (+)-tartaric acid, from which all absolute configurations are derived following the work of Bijvoet, Peerdeman, and Van Bommel (*Nature*, 1951, 168, 271), is similarly ambiguous being L- with respect to glyceraldehyde and D- with respect to serine

Equipotent Molar Ratios In order to express the activity of one drug relative to that of another I have used equipotent molar ratios, that is the number of molecules of one drug producing the same effect as one molecule of a standard drug If the compound is more active than the standard, the ratio will be less than one This may be confusing to people who feel that high activity should be indicated by a high figure but is more in accordance with what is actually done experimentally, less material is used if the compound is more active

I

Introduction

Approaches to pharmacology – Justification for a chemical approach to pharmacology – Validity of physical and chemical laws in biological systems – The mode of action of drugs on cells – Investigation of the mode of action of a drug – The drug-receptor complex and the kinetics of pharmacological reactions – Efficacy – Antagonists – A theory of drug action based on rates of combination with receptors – Factors affecting adsorbability – The nature and function of receptors – Enzymes – Enzyme kinetics – Conclusion – Classification of sites where drugs may act

Approaches to Pharmacology

'Pharmacology may be defined as the study of the manner in which the functions of living organisms can be modified by chemical substances' (A J Clark, 1937)

This study can be approached in different ways, depending upon the type and complexity of the tissue selected The effects of drugs* in man are those most likely to be of practical use Although experiments in man are almost invariably preceded by experiments in simpler structures, such as small animals, the understanding of the effects of chemical substances on such complex structures as these depends upon a knowledge of the normal working of these structures At this level pharmacology is an extension of physiology

The study of the effects of chemical substances on organisms which are parasites in man may also lead to discoveries of practical value in the treatment of diseases caused by such parasites Here pharmacology may be regarded as an extension of parasitology, particularly of bacteriology

At a more fundamental level, the effects of drugs on any tissue depend upon their actions on the cells of which that tissue is composed This demands a knowledge of the chemical processes occurring in the cells, and of the ways in which these processes are related to those occurring in other cells Here, then, pharmacology is an extension of biochemistry

Finally, all the effects of drugs are the consequence of the interactions of molecules of drug with molecules of the tissues, however complex, being studied At this level, therefore, pharmacology is an extension of chemistry in all its aspects, organic, inorganic, and physical

Pharmacology, therefore, draws upon physiology, bacteriology, biochemistry, and chemistry for essential basic information and most pharmacological laboratories contain workers from all these subjects The medical or therapeutic approach to pharmacology, however, is usually the dominant one because of its practical value and the need to train medical students

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* In this book the word 'drug' is used to describe any molecular species which may be of interest to the investigator, it does not refer only to substances which are known to be active

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Several books have been written on this aspect of pharmacology this book is an attempt to approach pharmacology at its most fundamental level, to discuss it as an extension of chemistry

Justification for a Chemical Approach to Pharmacology

The biggest difficulties in the way of regarding pharmacology as an extension of chemistry are the complexity of the processes occurring in living tissues and the impossibility of obtaining a precision in biological experiments comparable with that attainable in chemical experiments A great deal can be done by studying the actions of drugs on small pieces of tissue, or even on single cells, rather than on whole animals, and by a statistical treatment of the results Nevertheless, even simplified biological systems are far more complex than those met with in most types of physicochemical problem In the latter at least the identity of the molecular species involved is usually known, but in biological problems this is seldom true

Furthermore, interest in pharmacology, particularly in the extensive research work carried out in industry, lies chiefly in the production of compounds of potential therapeutic value Ideally such drugs could be discovered by a systematic study of the relationships between chemical structure and actions on simple isolated tissues, or even single cells A knowledge of such relationships between structure and action at all the important sites in the body should enable one to predict accurately the effect of a drug on the body as a whole In point of fact, with the present limited knowledge of biochemistry and pharmacology, it is not really possible to attempt this with any likelihood of success Most useful drugs have been discovered empirically as the result of 'screening' a large number of compounds in animals to see if they are useful and finding out why afterwards

A chemical approach to pharmacology can, therefore, be criticized on the grounds that it is based on over simplifications and is anyway unlikely to lead to results of any immediate practical value There is, however, much information about the apparent pharmacological properties of a lot of compounds In the circumstances it is natural to attempt to correlate chemical structure with biological activity even though such correlations can only be of any value if the compounds all act by the same mechanism There is, moreover, a certain amount of fundamental information about the mode of *action of some drugs*

A chemical approach to pharmacology can therefore be defended on the grounds that it is a rational approach to the subject which will not always be based on over simplifications, even if this is often so at present Further it may provide a logical attack on the problems of discovering new drugs Although it is true that many useful drugs have, in the past, been discovered empirically, an empirical approach has not usually been entirely haphazard, but has had some rational plan, even though the theories on which it has been based have often been shown to be quite wrong

Validity of Physical and Chemical Laws in Biological Systems

Before a chemical approach to pharmacology can be attempted it is necessary to show that the fundamental laws of physics and chemistry apply to atoms and molecules in systems composed of living matter

Clark (1933, 1937) has discussed one of the most striking features of pharmacology, the smallness of the amounts of a really active drug which may affect a really sensitive tissue Hunt (1918) recorded that detectable changes in the blood-pressure of a cat were produced by as little as 0·000,002 µg of acetylcholine per kg body weight This corresponds to 10^{-14} of a Mole, but is still equivalent to a very large number of molecules, 6×10^9, and these can be distributed over a very considerable area before the numbers become so small that the second law of thermodynamics is endangered The figures obtained by Hunt are exceptional Clark and White (1927) found the minimum effective dose to be 0 004 µg/kg in a similar experiment, and this order of magnitude seems more likely to be trustworthy

Bacterial toxins, however, are much more active and the results here cast some doubt about the validity of adopting a chemical approach to their action Botulinus Toxin type A has a molecular weight of 900,000 and the 'minimum lethal dose' for a mouse is 3.2×10^{-11} g (Van Heyningen, 1950) This is 3.6×10^{-17} Mole which should contain 2×10^7 molecules It has been calculated that 250 g of this material, if suitably administered, would suffice to kill the entire population of the world Botulinus Toxin type D is even more toxic Wentzel, Sterne, and Polson (1950) have obtained a purified material, of molecular weight about 1,000,000, which, when tested in mice, was found to contain 4×10^{12} 'minimum lethal doses' per mg of protein nitrogen For a nitrogen content of 15 per cent this gives a 'minimum lethal dose' of approximately

$$\frac{100}{15} \times \frac{1}{4 \times 10^{12}} \text{ mg}$$

$$= \frac{100}{15} \times \frac{1}{4 \times 10^{12}} \times \frac{1}{10^9} \text{ Mole}$$

$$= \frac{100}{15} \times \frac{1}{4 \times 10^{12}} \times \frac{6 \times 10^{23}}{10^9} = 1,000 \text{ molecules}$$

The claims of homeopaths are of a different order altogether Clark (1933) cites results of Konig (1927) reported to be obtained with concentrations of silver nitrate and lead nitrate as dilute as 1 in 10^{80}, and points out that this corresponds to about one molecule in a sphere with a circumference about equal to the orbit of the planet Venus Faith in such results clearly entails abandoning the laws of physics and chemistry, but otherwise, with possible reservations about bacterial toxins, it seems reasonable to assume that these are valid in the conditions prevailing in biological systems

The Mode of Action of Drugs on Cells

Although effective doses of even the most active substances contain large numbers of molecules, their action may be highly selective and confined to a very small area Clark (1933) estimated the percentage of the area of the cells of certain tissues which could be covered with drug molecules On the frog heart, for instance, a dose of 0 02 μg acetylcholine/g tissue caused a 50 per cent reduction in the rate of beating This corresponds to 10^{14} molecules (approximately) The dimensions of frog ventricle cells have been given as 131×9 microns and the surface area as 1,900 square microns ($= 1,900 \times 10^{-8} cm^2$) and volume 2,600 cubic microns ($= 2,600 \times 10^{-12} cm^3$) The number of cells per cm^3 or per g tissue is therefore $10^{12} \times 1/2,600 = 3 3 \times 10^8$, and the total surface area approximately $3 3 \times 10^8 \times 1,900 \times 10^{-8} = 6,000 cm^2$ This means that the number of molecules which are available for each cell is $10^{14}/3 3 \times 10^8 = 3 \times 10^5$ Clark assumed that each molecule could cover an area of 100 Å^2, and so the total area covered by 3×10^5 molecules would be $3 \times 10^5 \times 100 \times 10^{-8} = 0 3$ square microns, and this is only 0 3/1,900 of the area of the surface of a cell

Similar calculations showed that the drugs ouabain, acetylcholine, atropine, adrenaline, and histamine cannot possibly cover more than a fraction of the area of the cells of the tissues concerned (frog heart or rat uterus), whereas other compounds, such as caffeine and normal aliphatic alcohols (in the range heptyl to dodecyl), only produced effects when given in amounts which would suffice to form a monomolecular layer over the whole area of the cells

Similar calculations reveal the extremely selective uptake of bacterial toxins Botulinus Toxin is known to act on nerve-cells and the mouse contains approximately $2 5 \times 10^8$ such cells this indicates an average of 8 molecules of Botulinus Toxin Type A per cell and 1 molecule of Type D Toxin per 2,500 cells It would seem necessary to investigate further the site of action of these substances If only certain particular nerve cells need be affected to produce death, it is still possible that the numbers of molecules involved are sufficient to discuss their action in normal chemical terms

Although calculations of this sort can only be approximate, they indicate the likelihood of two types of drug action, one involving a large part, if not all, of the cell surface and the other involving only a very small part indeed The picture of a drug producing its effects by an action over a large part of the cell surface is consistent with the idea that it is acting by some physical or physicochemical process It may be having an effect on interfacial tension at the cell surface, dissolving preferentially in certain parts of the cell, having an action on cell colloids or affecting the membrane which surrounds the cell and which selectively gives passage to various ions Such a hypothesis was put forward at the end of the nineteenth century by Overton and Meyer to account for their results in research on anaesthesics

The picture of a drug producing its effects by an action only at a very small part of the cell surface is consistent with the idea that there are active spots, called receptors, on the cell surface and that the drug acts by forming a

complex with these This idea was suggested by Langley (1878, 1905), but is generally associated with the work of Ehrlich who used it as a basis for his research in chemotherapy (see, for instance, Albert, 1960)

Investigation of the Mode of Action of a Drug

It should, theoretically, be possible to decide whether a drug is acting by a physicochemical mechanism or at receptors by making calculations similar to those of Clark The necessary information about cell structure, however, is not always available and a decision about the type of action of a drug is frequently made instead by studying the activity of compounds chemically related to it

If there is no very obvious relationship between chemical structure and activity it is reasonable to suppose that the drugs are acting by a physicochemical process In these circumstances chemical structure will only be important in so far as it affects physicochemical properties

In the study of most drugs it is usual to find that there is a marked variation of activity with chemical structure In these circumstances it is reasonable to suppose that the drugs are acting on receptors because these receptors will themselves have a definite chemical structure in two or three dimensions and the action of the drug must depend upon its ability to fit

Physicochemical properties, however, may be extremely important in determining the rate of transport of a drug to its site of action on, or in, the cell and it is not always possible to distinguish clearly between drugs which are acting by a physicochemical mechanism and those which rely upon their physicochemical properties for transport but may act ultimately at receptors

The Drug-Receptor Complex and the Kinetics of Pharmacological Reactions

The combination of a drug with a receptor can be compared with the adsorption of a molecule at a catalyst surface An expression similar to the Langmuir adsorption isotherm relates the concentration of the drug, (A) with the proportion y, of receptors occupied in the reaction

$$\text{Drug} + \text{Receptor} \rightleftharpoons \text{Complex} \rightarrow \text{Breakdown} \rightarrow \text{Response}$$

The rate of formation of the complex will be $k_1(A)(1-y)$ and the rate of dissociation will be $k_2(y)$ where k_1 and k_2 are constants

At equilibrium $k_1(A)(1-y) = k_2(y)$ and hence $K(A) = \dfrac{y}{1-y}$ where K the affinity constant $= \dfrac{k_1}{k_2}$ When 50 per cent of the receptors are occupied $(A) = \dfrac{1}{K}$

Note that K is an affinity constant, some workers (e g Ariens 1954) use the dissociation constant, which is the reciprocal of this and can be compared with the Michaelis–Menten constant used by enzymologists (see page 20)

If the biological response is directly related to the proportion of receptors occupied the graph of dose against response will be a hyperbola In view of experimental errors, however, the mathematical relationship between dose

and response cannot, with confidence, be deduced from the shape of the curve Clark (1933) showed that the differences between the hyperbola,

$$KA = \frac{y}{1 - y}, \ (K = 1),$$

the exponential curve, $Ky = \log(bA + 1), \ (K = 0.0166, \ b = 5.3),$

and the parabola, $KA^n = y, \ (K = 49, \ n = 0.5),$ were much smaller than the errors in the most accurate biological experiments which had been performed at that time The second formula represents the empirical Weber–Fechner 'Law' and the third an adsorption isotherm of the Freundlich type

Although the formation of the drug receptor complex is generally regarded as being governed by the Langmuir Adsorption Isotherm, the pharmacologist usually plots the logarithm of the dose against the effect and finds that this graph is linear over a considerable range This process is much more convenient than plotting the dose against

$$\frac{\text{Percentage effect}}{100 - \text{Percentage effect}},$$

though the logarithm of this is the 'logit' function, which is available in tables The graph of the logarithm of the dose against the logit of the response might, therefore, be expected to be linear, but the expression

$$\frac{\text{Percentage effect}}{100 - \text{Percentage effect}}$$

will only be the same as $\frac{y}{1 - y}$ if the biological response is directly proportional to the proportion of receptors occupied This was assumed by Clark (1937), although he himself says the assumption seems improbable, and such attempts as have been made to test the validity of the assumption (see below) indicate that it is not justified If it were true, the concentration of a drug which produced half the maximal response would be equal to the reciprocal of the affinity constant, this value has, in fact, sometimes been used as an index of the affinity of the drug for the receptors

Efficacy

In experiments with acetylcholine and tetramethylammonium, Clark and Raventos (1937) showed that although acetylcholine was about 1,000 times as active as tetramethylammonium, the effects of equiactive doses of the drugs were antagonized to the same extent by an antagonist (Table I 1) From these results there emerged the idea of the all or none nature of the drug-receptor complex, i e that the complex between a drug and a receptor was either completely effective or completely ineffective If it were effective, the compound would be an agonist, if it were ineffective, the compound would be an antagonist In either situation the activity of the drug (as an agonist or as an antagonist) would depend only on its adsorbability or affinity constant, K

This idea was supported by the observation that the effects of acetylcholine and tetramethylammonium were additive If the effects of x acetylcholine were the same as y tetramethylammonium (y would be about 1,000 times x), then these were also produced by $x/2$ acetylcholine plus $y/2$ tetramethylammonium together If the complex produced by one drug were less effective than the complex produced by the other, it would occupy receptors which could more profitably be occupied by the other drug, so the effects would not be additive

Although these conclusions are perfectly valid for acetylcholine and tetramethylammonium on the tissues listed in Table I 1, they cannot be

TABLE I 1

Antagonism of the Actions of Acetylcholine and tetramethylammonium

	Preparation					
	Rat intestine		Frog auricle		Frog rectus	
Agonist Antagonist	Ach	Me₄N⁺	Ach	Me₄N⁺	Ach	Me₄N⁺
Atropine + n OctNMe₃	−8 1	−7 9	−8 3	−7 7	−4 2	−3 8
+ n OctNMe₃	−5 5	−5 0	−5 0	−4 6	−4 1	−4 0
n Bu₄N 'Curarine'	−3 6	−3 7	—	—	−3 2	−3 0
	—	—	−5 1	−4 5	−6 8	−6 5

Figures indicate the logarithm of the molecular concentration of antagonist which necessitated multiplying the concentration of agonist by 10 in order to keep the biological effect constant, these are in effect, values of $-pA_{10}$ (page 43) The value for an antagonist on a particular tissue is considered to be independent of the nature of the agonist, compare, for example, the values for atropine and acetylcholine (Ach) with those for atropine and tetramethylammonium, −8 1 and −7 9 on rat intestine, −8 3 and −7 7 on the frog auricle, and −4 2 and −3 8 on the frog rectus

Clark and Raventos (1937)

extended universally From further experiments, Ariens (1954) and Stephenson (1956) have suggested that the activity of a drug depends not only on its affinity (adsorbability) but on another property termed 'intrinsic activity' (Ariens) or 'efficacy' (Stephenson), this factor being a measure of the effectiveness of the drug-receptor complex

Ariens (1954) considers the situation in which the response depends directly upon the intrinsic activity, α, and the proportion of receptors occupied, y In the experiments of Clark and Raventos α for the antagonists is zero, whereas α for tetramethylammonium is the same as that for acetylcholine, which is taken as unity There are, however, substances with an intermediate intrinsic activity, which are called dualists (Ariens) or partial agonists (Stephenson) Their low intrinsic activity is revealed by their ability to antagonize compounds of higher intrinsic activity They fail to act additively,

presumably because they occupy receptors which could be more profitably occupied by the drug with the higher intrinsic activity Furthermore, such drugs, however high the concentration tested, do not produce the maximal response of which the tissue is capable, for if the response varies with αy, and the maximal response, R_{Max}, is obtained when $y = 1$ and $\alpha = 1$, the response R to a dualist (intrinsic activity α) obtained with any concentration sufficient to saturate the receptors ($y = 1$) will be $R/R_{Max} = \alpha$ Ariens therefore estimates intrinsic activity by determining this fraction experimentally

In his papers (e g Ariens, 1954, Ariens and De Groot, 1954, Ariens and van Rossum, 1957) will also be found estimates of the affinity constant, K, based on the determination of the concentration which causes half the maximal response (in the case of a dualist, half the maximal response of which the drug is capable) The logarithm of the reciprocal of this concentration is called pD_2, following a convention originally proposed for antagonists by Miller, Becker, and Tainter (1958, see page 193, D stands for dilution)

This treatment involved two assumptions, that the response is directly proportional to the fraction of the total receptor population occupied, and that no agonist can have an intrinsic activity greater than unity Stephenson avoids making these assumptions by introducing another term, the biological stimulus, S, where $S = ey$, e being the efficacy, which can have any positive value from zero upwards, and y being the proportion of receptors occupied No assumption is made about the relationship between S and the biological response, though it is supposed that equal values of S will give equal biological responses as an arbitrary standard, a 50 per cent response is regarded as being produced when $S = 1$ Stephenson further suggests that it may be incorrect to suppose that a maximal response is only produced when all the receptors are occupied He postulates that there may be 'spare' receptors and that, in consequence, a drug of high efficacy may give a maximal response when only a small proportion of the total receptor population is occupied

Following from the convention, $S = 1$ for a 50 per cent maximal response, a partial agonist which can only produce a 50 per cent maximal response, even when all the receptors are occupied, has an efficacy of 1, for $S = ey$ (and $y = 1$)

From results with butyltrimethylammonium (on the guinea pig ileum) Stephenson was able to obtain experimentally some idea of how the response was related to the biological stimulus for this particular drug and tissue If the proportion of receptors occupied by butyltrimethylammonium is only a small fraction of the total, this proportion, y, will vary directly with the concentration of the drug for,

$$K(A) = \frac{y}{1 - y} \quad \text{and} \quad 1 - y \to 1$$

and $S = ey$, so S will vary directly with (A) The relationship between response and stimulus can then be obtained by plotting response against dose, where the dose interval between no response and 50 per cent response is taken as

1 unit (Fig I 1) This curve is **not linear** From it and from the equations $S = ey = \dfrac{eK(A)}{1 + K(A)}$ Stephenson was able to construct a series of graphs showing the variation of percentage response with concentration of drug for different values of e but with the same value of K These (Fig I 2) are quite different from a similar family of curves calculated on the assumptions of Ariens (Fig I 3), one of the chief differences being that in the latter, once the drug is capable of producing a maximal response, activity can only
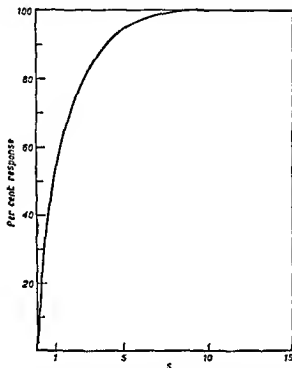
FIG I 1 *Relationship between stimulus S, and percentage response for n butyl trimethylammonium on the guinea pig ileum This is obtained by plotting the per-centage response against the dose when the latter is expressed as a multiple of the difference between the amount producing no response and the amount producing half the maximal response (Stephenson 1956)*

increase if affinity increases in the former it can increase because efficacy rises without an increase in affinity

If the postulate of the existence of spare receptors is correct (and this seems likely to be so) the affinity constant K cannot be obtained by measuring the concentration of agonist which produces 50 per cent of the maximal response It can, however, be arrived at in certain circumstances For an active agonist the expression

$$S = \frac{eK(A)}{1 + K(A)}$$

reduces to $S = eKA$, because KA will be small (page 8) Now let an experiment be performed in which the response (I) to a concentration (P) of a
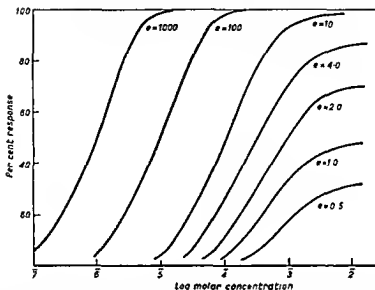
FIG. 1 2. *Relationship between percentage response and molar concentration of agonists with the same affinity constant ($K = 10^2$) but different efficacies. The curves were obtained by calculating the proportion of receptors occupied at a particular concentration of agonist, multiplying by the efficacy to obtain the stimulus, S, and reading off the percentage response this should produce from the curve shown in Fig. I.1. (Stephenson, 1956)*
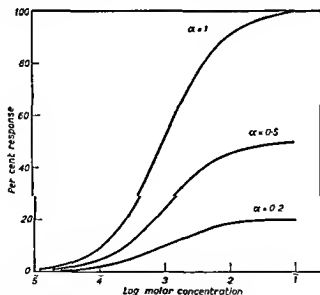
FIG. 1 3. *Relationship between percentage response and molar concentration of agonists with the same affinity constant ($K = 10^2$) but different intrinsic activities (efficacies), calculated on the assumption that the response is directly proportional to the stimulus.*

partial agonist (efficacy e_p) is matched by a concentration (A_1) of an active agonist (efficacy e_a, affinity constant K_a), and a response (II) to (P) plus (A_3) of agonist is matched by (A_2) of agonist alone (Fig 1 4) If the partial agonist occupies a proportion x of the receptors, the response I is produced by a stimulus $S_1 = e_p x = e_a K_a (A_1)$
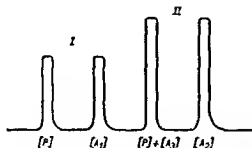
FIG 1 4   *Determination of the affinity constant of a partial agonist   The response (I) to [P] of a partial agonist is matched by that to [A_1] of an active agonist and the response (II) to [P] + [A_3] of the active agonist is matched by that to [A_2] of the active agonist alone (Stephenson, 1956)*

The response II is produced by a stimulus

$$S_2 = e_a K_a (A_2) = e_p x + e_a K_a (A_3)(1 - x),$$

assuming values of S are additive and that the active agonist occupies only a negligible proportion of receptors, whereas the partial agonist occupies a significant proportion

Hence, $e_a K_a (A_2) = e_a K_a (A_1) + e_a K_a (A_3)(1 - x)$

or $$1 - x = \frac{A_2 - A_1}{A_3}$$

Values of x for different concentrations of partial agonist can be obtained and the true concentration producing 50 per cent occupation of the receptors determined

Values of the affinity constant, K, obtained in this way are not the same as those obtained from the concentration producing half the maximal response   For instance, Stephenson, using the above procedure found the affinity constant for n heptyltrimethylammonium (a partial agonist) on the guinea pig ileum to be $4 1 \times 10^4$, the concentration producing half the maximal response of which the drug is capable was 5×10^{-6} M, which would give an affinity constant of 2×10^5

When discussing relationships between chemical structure and activity, it should be important to distinguish between effects on efficacy and effects on adsorbability   It is, however, usually impossible to relate changes in chemical structure with changes in efficacy because the efficacy of a drug is seldom known   Relationships between chemical structure and adsorbability,

however, should be relatively easily arrived at Although the true affinity constants of agonists are rarely determined, a considerable amount of information about the influence of structure on adsorbability can be obtained from antagonists, whose efficacy is zero

Antagonists

If an additional molecular species is present which is capable of combining with the receptors but forms a complex which produces no biological stimulus, the situation can be written (Gaddum, 1937)

$$\text{Agonist} + \text{Receptor} \rightleftharpoons \text{Complex} \rightarrow \quad \rightarrow \text{response}$$
$$\text{Antagonist} + \text{Receptor} \rightleftharpoons \text{Complex}$$
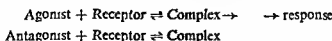
If the agonist molecules, in concentration (A), occupy a proportion, y, of the receptors, and if the antagonist molecules, in concentration (B), occupy a proportion z, we can write (as on page 5),

$$K_A = \frac{y}{(A)(1-y-z)} \quad \text{or} \quad y = K_A(A)(1-y-z)$$

where K_A is the affinity constant for the agonist and the receptor

$$K_B = \frac{z}{(B)(1-y-z)} \quad \text{or} \quad z = K_B(B)(1-y-z)$$

where K_B is the affinity constant for the antagonist and the receptor

Dividing the two, $\dfrac{y}{z} = \dfrac{(A)K_A}{(B)K_B}$,

But

$$z = \frac{(1-y)(B)K_B}{1+(B)K_B}$$

and hence

$$(A)K_A = \frac{y}{(1-y)}(1+(B)K_B)$$

When no inhibitor is present this becomes $\frac{y}{1-y}$ as on page 5, if the biological response to a concentration, (A), of agonist in the presence of a concentration, (B) of antagonist is the same as that to a concentration (a) of agonist alone,

$$\frac{(A)}{(a)} = 1 + (B)K_B$$

When $(A)/(a)$ is 2, that is, the concentration of the antagonist necessitates doubling the dose of agonist in order to keep the effect constant,

$$(B)K_B = 1, \quad \text{or} \quad (B) = 1/K_B$$

This method of determining K_B makes no assumptions about the relationship between the biological stimulus, or proportion of receptors occupied, and the size of the response, because the size of the response is kept constant The value of (B) should be constant as it is equal to the reciprocal of the

affinity constant This is the principle underlying the use of pA_x to express antagonist activity (Schild, 1947) discussed in Chapter II (page 43) The value pA_2, the logarithm of the reciprocal of the concentration of antagonist which necessitates doubling the concentration of agonist in order to keep the effect constant, is equal to the logarithm of K, the affinity constant for the antagonist

Discussion of the relationships between chemical structure and affinity based on the determination of pA_2 values is therefore justifiable to a much greater extent than is possible when affinity is based on the determination of pD values for agonists (page 8) The antagonism considered here, however, is a particular type, referred to as competitive antagonism, in which the two molecular species, agonist and antagonist, compete for the same receptors The antagonism is completely reversible, an increase in the concentration of agonist will overcome the effect of the antagonist and vice versa The degree of antagonism produced (as measured by A/a) depends on the concentration of the antagonist and its association constant, K_B One characteristic of this type of antagonism, therefore, is that the log dose-response curves in the presence of the antagonist are parallel to those for the agonist alone, being merely displaced towards higher concentrations by an amount equal to log A/a one thus obtains a family of parallel curves representing the variation of response with the logarithm of the dose of the agonist in the presence on different concentrations of antagonist (Fig 15) Not all antagonists behave in this way Some, for example, react with the receptors to produce a complex which is very stable and such antagonists are virtually irreversible They can only be removed by introducing yet another molecular species, with which the reacting group on the antagonist combines even more readily The collision of this reactivator molecule with the antagonist-receptor complex will then result in the transfer of the group to the reactivator and this product subsequently diffuses away from the receptor

Another type of antagonist with different characteristics may act like an agonist at the receptors but fail to produce exactly the right type of stimulus In consequence the biological processes which culminate in the response become blocked at some point beyond the first stage Such an antagonist, like the irreversible ones discussed above, may only act after a considerable time lag, whereas the effects of competitive antagonists appear rapidly, being dependent only on rates of diffusion for the achievement of equilibrium In the determination of pA_2 with histamine and guinea-pig ileum, for example, the antagonism is maximal within about 15 minutes indicating that equilibrium takes only this time to become established

It is possible to imagine a number of situations in which antagonism is other than competitive Many such have been considered in detail by Ariens, van Rossum, and Simonis (1957) It may be questioned how far these hypotheses can be tested experimentally in view of the inaccuracies inherent in quantitative pharmacological measurements, but one situation, which has been called non competitive antagonism (Schild, 1954), will be discussed
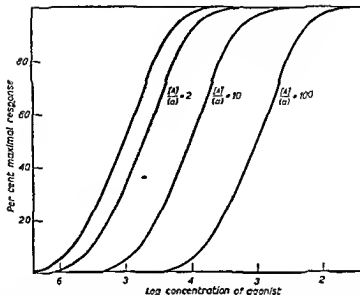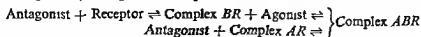
FIG 1 5   *Effects of a competitive antagonist graphs of log concentration of agonist against percentage of the maximal response in the presence of different concentrations of antagonist*

For an antagonist with an affinity constant of 10^3, the curve where $[A]/[a] = 2$ (displaced 0 3 log dose units to the right of the curve in the absence of antagonist) would be produced by a concentration of 10^{-3} M, for $[A]/[a] = 10$ by a concentration of 9×10^{-3} M, and for $[A]/[o] = 100$ by $9 9 \times 10^{-2}$ M The actual agonist used should be immaterial, the graph in this figure is that for the compound with an efficacy of 100 and affinity constant of 10^3 shown in Fig 1 2 Similar parallel curves will be obtained if the proportion of receptors occupied, y, is plotted instead of the corresponding response derived from Fig 1 1

Consider the situation where the formation of the complex by the antagonist depends only on the proportion of receptors not occupied by antagonist, thus

$$K_B = \frac{z}{(1 - z)(B)},$$

where z is the proportion of receptors occupied by antagonist and (B) is the concentration of antagonist This is different from the competitive situation in that the proportion, y, of receptors occupied by the agonist does not matter for the antagonist, possibly because the antagonist reacts with these to form a complex, Agonist–Receptor–Antagonist, according to the scheme

Agonist + Receptor $\rightleftharpoons$ Complex AR

Antagonist + Receptor $\rightleftharpoons$ Complex BR + Agonist $\rightleftharpoons$ } Complex ABR
Antagonist + Complex AR $\rightleftharpoons$ }

The same situation would also arise if the stability of the complex BR were very much higher than that of the complex AR, so that it virtually did not break down The further combination of molecules of such an 'irrever-

sible' blocking agent with *receptors* would depend only upon the proportion, $1 - z$, of receptors not already occupied

For the combination of agonist and receptor,

$$K_A = \frac{y}{(1 - y - z)(A)}$$

Hence

$$y = (A)K_A - y(A)K_A - z(A)K_A$$
$$= \frac{(A)K_A(1 - z)}{1 + (A)K_A}$$

But

$$z = (B)K_B - z(B)K_B$$
$$= \frac{(B)K_B}{1 + (B)K_B}$$

So

$$y = \frac{(A)K_A}{1 + (A)K_A}\left(1 - \frac{(B)K_B}{1 + (B)K_B}\right)$$
$$= \frac{(A)K_A}{1 + (A)K_A}\left(\frac{1}{1 + (B)K_B}\right)$$

When $B = 0$, this reduces to the expression for the agonist alone For different values of (B), however, the log dose-effect curves are not parallel but decline progressively (Fig 1 6) If a maximal response is obtained when
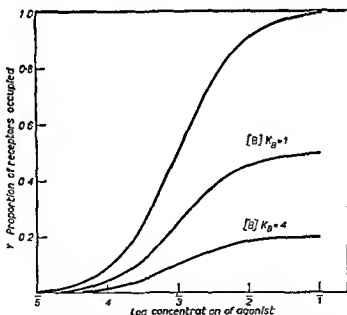
FIG 1 6   *Effects of a non competitive antagonist graphs of log concentration of agonist against proportion of receptors occupied in the presence of different concentrations of antagonist The top graph is for an agonist (affinity constant, 10^3) alone The middle and lower graphs are for agonist in presence of antagonist ($10^{3} M$ and $4 \times 10^{3} M$, respectively, if $K_B = 10^3$) If there are 'spare' receptors the graphs will be similar to those in Fig 1 2 they will appear to be parallel until the block has developed to the point at which the proportion of receptors still unblocked is less than that which can produce a maximal response*

y is less than 1, i e if there are spare receptors, it will be seen that, when $(B)K_B$ is small, this type of antagonism will appear reversible, for higher values of $(B)K_B$, the block will not be reversed by the addition of more agonist This condition has been described as 'unsurmountable' by Gaddum, Hameed, Hathway, and Stephens (1955)

To obtain some sort of estimate of the activity of such an antagonist, Schild (1957) and Ariëns and Van Rossum (1957) have suggested the use of the concentration of antagonist which reduces a maximal response to the agonist to a half maximal response The logarithm of the reciprocal of this concentration is called pA$_x$ (Schild) or pD$_2'$ (Ariëns and Van Rossum) If the response is directly proportional to the fraction of receptors occupied, this value would have some significance, for then

$$\frac{(A)K_A}{1 + (A)K_A} = y \text{ (for a maximal response, this may not be unity if there are spare receptors)}$$

$$\text{and } \frac{(A)K_A}{1 + (A)K_A}\left(\frac{1}{1 + (B)K_B}\right) = y/2 \text{ (for a half maximal response)}$$

Hence, dividing, $2 = 1 + (B)K_B$

and $\log K_B = -\log (B) = \text{pA}_x$

As it seems unlikely that it is correct to assume that the response varies linearly with the proportion of receptors occupied, pA$_x$ or pD$_2'$ cannot be regarded as having any absolute significance, and it is questionable how far such values may be used as a basis for the discussion of the variation of affinity with chemical structure

A Theory of Drug Action Based on Rate of Combination with Receptors

The interaction of a drug with a receptor involves three steps, the adsorption of the drug, some process or reaction consequent upon the formation of the complex, and lastly desorption of the drug In the discussion of the mechanism of action of drugs so far it has been assumed that the response is a function of the proportion of receptors occupied by agonist molecules This assumes that biological stimulus will continue to be produced all the time that the drug and receptor are combined The receptors can, therefore, be compared with the keyboard of an organ, the pipes of which will continue to speak so long as the note is depressed

Paton (1961) has suggested that the stimulus may depend upon the rate of combination with the receptors and that a situation analogous to a piano might be more correct On this theory an active compound would have to form a complex which was rapidly broken up and the rate-constant for the break up of the complex would be analogous to 'efficacy' or 'intrinsic activity' This 'rate' theory of drug action leads to a relationship between biological stimulus and the concentration of drug which is very similar to that obtained on the 'occupation' theory At equilibrium the rate of association of drug molecules with receptors will be $k_1(A) (1 - y)$ and will equal the rate of dis-

sociation, $k_2(y)$, where k_1 is the rate-constant for association, k_2 is the rate-constant for dissociation, (A) is the concentration of drug, and y is the proportion of receptors occupied As on page 5, $y = \dfrac{(A)}{(A) + k_2/k_1}$ and hence the rate of association $= \dfrac{k_2(A)}{(A) + k_2/k_1}$

Paton suggests that this rate is directly related to the response, but there is no reason why this should be so, it could, conceivably, only be comparable with the 'biological stimulus' as described by Stephenson (1956)

From the dose-response curves it is impossible to distinguish between the two theories Paton showed that the results for histamine on the guinea-pig ileum, for example, would fit a curve of the type $y = \dfrac{x}{x + 0\,6}$, especially if a particular type of lever (termed 'auxotonic') were used to record the contractions of the muscle This, in itself, does not prove anything (particularly in view of Clark's remarks referred to on page 6), but with an auxotonic lever the contraction of the guinea pig ileum invariably 'fades' after a dose of agonist, i e it declines immediately after it has reached its peak This would be consistent with the decline in the rate of combination of drug with the receptors which should occur when a high proportion of these has become occupied immediately after the drug has been added On the occupation theory there is no reason why it should occur If other types of lever are used, however, 'fade' is often not seen, which suggests that it might have some other explanation

One other phenomenon which the rate theory might explain conveniently is 'tachyphylaxis' or 'desensitization', that is, the insensitivity of a tissue immediately after it has been treated with a high dose of agonist If the receptors have just been saturated with drug molecules the rate of combination would necessarily be very low Tachyphylaxis, however, is often unspecific, a large dose of acetylcholine renders the guinea pig ileum insensitive to histamine and 5 hydroxytryptamine as well as to acetylcholine It is known that these substances act at different receptors on the tissue because there are drugs which will block specifically the actions of only one agonist and do not affect the others This suggests that tachyphylaxis produces some general change in the tissue, rather than a particular effect at one type of receptor

The 'rate' theory remains a possibility about which it will be very difficult to decide, particularly if it is modified so that, as with the 'occupation' theory, the rate of occupancy is considered to determine the 'biological stimulus' rather than directly to determine the response It is attractive in that it takes into consideration the third stage in the action of a drug, desorption, which is largely neglected on the occupation theory It would also explain why antagonists are usually bigger molecules than agonists and why many antagonists are active in much lower concentrations than agonists, though this can also be explained by the occupation theory Perhaps it is fortunate that both theories lead to similar relationships between drug concentration and biological stimulus and it is conceivable that they are not mutually

exclusive It could well be that with most agonists the proportion of receptors occupied (and the efficacy of the compound) determines the biological stimulus, but that with others the slow break up of the complex reduces the efficacy of the compound There is no reason why efficacy should not depend upon a number of independent factors, of which the stability of the complex and the ability to promote some change or reaction might be examples The situation may indeed be analogous to a piano, but even with a piano there is some degree of sustaining, the vibrations are not damped instantaneously

Factors Affecting Adsorbability

The adsorbability of a drug will depend upon its ability to fit the receptor and on any possible interaction between groups in the drug and groups in the receptor Such groups in the drug are accordingly important in determining its biological activity and are termed pharmacodynamic groups, in this book the groups in the receptor with which they interact are termed receptor groups (the whole receptor being called a receptor unit)

Four types of link binding drug and receptor may be considered Arranged roughly in order of strength, these are

1. Covalent bonds
2. Coulombic attraction between positive and negative charges (not necessarily involving whole units of charge)
3. Hydrogen bonds
4. Van der Waals' forces

The energy of covalent bonds varies from around 20 to 100 kcal/mole and a single such link between drug and receptor could result in the formation of a very stable complex Even a weak covalent bond, such as binds arsenical drugs (in the form of arsenoxides) to thiol groups –

$$-As{=}O + \begin{matrix} HS- \\ HS- \end{matrix} \longrightarrow -As{\Big<}\begin{matrix} S- \\ S- \end{matrix}$$
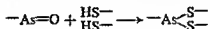
– gives a complex which does not dissociate to any appreciable extent in biological conditions A bond whose strength is more than 10 kcal/M is unlikely to break up at body temperature Such links can, however, be broken by the addition of other compounds containing —SH groups to which the arsenic atom becomes transferred

The force between positive and negative charges varies with the size of the charge and the square of the distance between the charged centres In the most favourable conditions, when two whole units of charge are involved and come close together, the bond energy is around 5 kcal/M, which is about the same as that of a hydrogen bond The interaction between partial charges on polar groups is less than this, as is also the Van der Waals' bond energy The Van der Waals' bond energy may be of the order of 0 5 kcal/M, but depends very greatly on the atoms concerned being able to come close together (the bond strength varies inversely as the 7th power of the distance between the atoms)

Any one of these last three types of bond – ionic, hydrogen, and Van der Waals' – could not by itself give rise to a very stable complex between drug and receptor If, however, interaction between drug and receptor can take place at a number of points, at which any of these types of bond may be involved, the multiple forces binding drug and receptor could give rise to a complex with considerable stability Io these circumstances the formation of the drug receptor complex is, nevertheless, likely to be a reversible process and the stability of the complex will depend very greatly on the drug and receptor being able to fit closely together

The Nature and Function of Receptors

Although it is possible to interpret the actions of drugs at receptors without considering these as more than hypothetical structures, it is natural to consider what such structures may be and what they may do, especially as any information about this should lead to a fuller understanding of the mode of action of drugs

The receptors affected by acetylcholine belong to a special part of the cell concerned with its function, namely the apparatus responsible for initiating contraction in muscle cells or for transmitting nerve impulses in ganglion cells (see Chapter IV) The biological response involves physical changes in the permeability of the cell membrane, in the polarization of the cell membrane, in movements of ions through the cell membrane and, in muscle, in the length of the whole cell These events may be a purely physical consequence of the biological stimulus arising from the drug receptor complexes These complexes may physically alter the membrane, perhaps changing its shape or the size of pores in it, and this physical change may produce the response Another possibility, however, is that the drug receptor complexes activate or inactivate some enzyme system or systems, and the physical events follow as a consequence of these enzyme changes Some of the events, such as changes in polarization, might even be incidental and not directly connected with the biological response at all, although this seems rather unlikely

The picture of drug receptor complexes acting by physical means is not particularly helpful as there are few mechanisms with which this can be compared The characteristics of enzymic processes, on the other hand, are well known and some drugs appear definitely to be acting through such mechanisms (the arsenical drugs, for example, appear to inhibit respiratory enzymes, see review by Albert, 1960)

Enzymes

Enzymes are proteins which catalyse reactions occurring in biological systems Most enzymic processes involve the alteration (e g by hydrolysis, oxidation, or reduction) of one particular molecular species called the substrate The reaction takes place at a point on the enzyme surface called the 'active spot' Experimental evidence (see, for example, Dixon and Webb, 1958) indicates

that the number of active spots per protein molecule is low, being of the order of unity or, at the most (so far observed), ten

Enzymes can be divided into three groups

1 Those which have absolute specificity, that is, which affect only one particular substrate For example, arginase, the enzyme which effects the hydrolysis of S arginine, is absolutely specific The substrate must be arginine, the enzyme will not effect the hydrolysis of substances even extremely closely related to arginine The enzyme is also stereospecific, only the S form is affected ($\pm$) arginine is converted into a mixture of R-arginine and S ornithine

2 Those which have group specificity Many enzymes do not distinguish between substrates which are closely related chemically to each other For example, acetylcholine esterase hydrolyses acetyl β-methylcholine and, less rapidly, acetyl-α methylcholine and triacetin as well as acetylcholine itself The enzyme, however, does not hydrolyse butyrylcholine

3 Those which have low specificity These are not very common, but there are lipases (enzymes which effect the hydrolysis of fats) which belong to this class They affect fats generally regardless of the nature of the fatty acid part of the molecule

The reaction may involve another molecular species (e g water, oxygen, or a hydrogen acceptor) besides the enzyme and the substrate The enzyme may be selective and the reaction only proceed if this additional molecular species is also of a particular structure, an oxidation, for example, with an enzyme may only proceed if a particular hydrogen acceptor is present This additional species is termed a coenzyme In certain enzymes an appropriate coenzyme occurs naturally with the enzyme, being held quite firmly to the surface of the protein but separable from it by dialysis In these circumstances the 'built in' coenzyme is called a prosthetic group and the enzyme protein is called the apo enzyme

Enzyme Kinetics

The adsorption of the substrate at the active spot on an enzyme is exactly analogous to that of a drug at a receptor, already discussed

$$\text{Substrate} + \text{Enzyme} \rightleftharpoons \text{Complex} \rightarrow \text{Enzyme} + \text{Products}$$
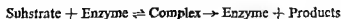
The rate of formation of the complex $= k_1(s)(e - p)$, where s is the substrate concentration, e the enzyme concentration, and p the concentration of the complex The rate of dissociation of the complex $= k_2 p$ At equilibrium $k_1 s(e - p) = k_2 p$ The procedure, due to Michaelis and Menten then writes a third constant $K_s = \dfrac{(e - p)s}{p}$ This constant, known as the Michaelis-Menten constant is the dissociation constant of the enzyme-substrate complex and it should be noted that this is k_2/k_1, whereas the 'affinity constant'

of the pharmacologist is k_1/k_2 This latter expression is also used by some workers to denote the affinity of enzyme and substrate and so has the value $\frac{1}{K_s}$

The Michaelis-Menten equation can be rewritten

$$K_s p = es - ps, \quad p = \frac{es}{K_s + s}$$

The reaction velocity, v, is assumed to depend on the rate of breakdown of the complex, hence $v = kp$, where k is the velocity constant, therefore $v = \frac{ke}{1 + K_s/s}$. When s is large, this becomes $V = ke$, where V is the maximal
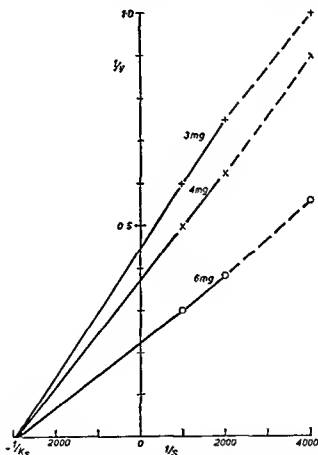
FIG 1 7 *Graphs of $1/v$ against $1/s$ for the hydrolysis of acetylcholine by acetyl cholinesterases of dog s caudate nucleus v is measured in ulitres of carbon dioxide evolved per minute Although the actual rate depends upon the amount of enzyme present estimates of K_s are not dependent on this and agree closely ($1/K_s = 2\,800$), especially if the results obtained with the lowest concentration of substrate are not given full weight (these especially in the experiments with only 3 mg tissue/flask, are at about the limit of the sensitivity of this particular apparatus) In these experiments the substrate concentration is too low for there to be inhibition by excess substrate (Chapter VIII) (Results obtained by Hamilton, 1961)*

velocity, obtained when the enzyme is saturated with substrate The velocity at a lower degree of saturation can therefore be written $v = \dfrac{V}{1 + K_s/s}$ and when $v = V/2$, $s = K_s$ (The equation is often also written in the form $(V - v)(K_s + s) = VK_s$)

The adsorbability of a substrate at the active spot of an enzyme can, therefore, be determined simply and accurately and expressed as the reciprocal of the Michaelis-Menten constant, K_s In addition to the method already mentioned, plotting the graph of the rate of the reaction against the substrate concentration and observing the concentration at which the rate is half-maximal, the Michaelis-Menten constant can be determined by a number of other methods (see, for instance, Dixon and Webb, 1958) In the procedure of Lineweaver and Burk (1934), $1/v$ is plotted against $1/s$,

since
$$v = \frac{V}{1 + K_s/s}$$

$$1/v = 1/V + K_s/sV,$$

so the graph should be a straight line with intercepts of $1/V$ and $-1/K_s$ and with a slope of K_s/V (Fig I 7)

In the method of Augustinsson (1948), v is plotted against v/s,

since
$$V = v + K_s v/s,$$

this should be a straight line with a slope of $-K_s$ and intercepts of V and V/K_s (Fig I 8)

The value of the Michaelis-Menten constant is much more informative than the pharmacological affinity constant because, in the enzymic experiments, the response, i e the rate of the reaction, should be directly related to the number of active spots occupied The velocity constant, k, for the breakdown of the complex, however, varies from one substrate complex to another (depending on the nature of the substrate) and can be likened to the efficacy

The effect of an inhibitor can be calculated in a way analogous to that for receptors (page *12*), because dissociation constants, rather than association constants, are used in enzymology, the equation

$$(A)K_A = \frac{y}{1 - y}(1 + (B)K_B)$$

becomes
$$s/K_s = \frac{y}{e - y}(1 + I/K_I),$$

where I is the concentration of inhibitor and K_I is the inhibitor constant, the dissociation constant of the complex formed by the enzyme with the inhibitor

Accordingly $$es/K_s - \jmath s/K_s = y(1 + I/K_i)$$

$$y = \frac{es/K_s}{s/K_s + 1 + I/K_i}$$

$$= \frac{e}{1 + K_s/s(1 + I/K_i)}$$

and the rate of reaction, $$v = \frac{V}{1 + K_s/s(1 + I/K_i)},$$

where V is the maximum velocity, obtained when no inhibitor is present and the enzyme is saturated with substrate

When $I = 0$, this reduces to $v = \dfrac{V}{1 + K_s/s}$
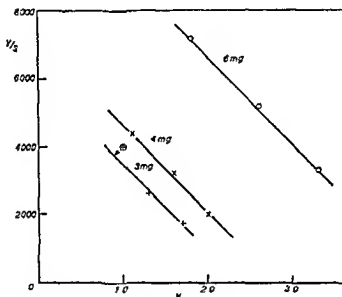
FIG 18 *The graphs of v against v/s for the same results as in Fig 17 If the point for the lowest concentration of substrate and the smallest amount of enzyme is neglected, the slopes of the lines are very similar and independent of the actual amount of enzyme present, they indicate a value of 2,500 for 1/K_s*

The equation for the inhibited reaction can also be written

$$1/v = 1/V + K_s/sV + \frac{K_s I}{sK_i V}$$

consequently the graph of $1/v$ against I is a straight line If the experiment is repeated with a second concentration of substrate, s, a second line will be obtained, and Dixon (1953) has shown that this should intersect the first line at the point where $I = -K_i$ (Fig 19) At the point of intersection, $1/v$ and I will be the same for both sets of experiments and so will V be also,

B

because the same amount of enzyme is used in all the experiments Accordingly,

$$\frac{K_s}{sV} + \frac{K_s I}{sK_i V} = \frac{K_s}{s'V} + \frac{K_s I}{s'K_i V}$$

or

$$\frac{1}{s}(1 + I/K_i) = \frac{1}{s'}(1 + I/K_i)$$

and because s does not equal s', $(1 + I/K_i)$ must be zero, i e $I = -K_i$
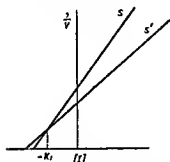
FIG 1 9   Graphs of $1/v$ against inhibitor concentration, $[I]$, for a competitive antagonist, the two lines represent results with different concentrations of substrate, s and s', and should intersect when $I = -K_i$
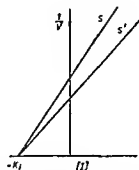
FIG 1 10   Graphs of $1/v$ against inhibitor concentration, $[I]$, for a non competitive antagonist of the type discussed on page 14, the two lines represent results with different concentrations of substrate, s and s', and should intersect when $I = -K_i$ and $1/v = 0$

If the inhibition is non-competitive and of the type described for pharmacological receptors on page 14, in which the formation of the complex by the inhibitor is independent of the formation of complexes by the substrate,

$$K_i = \frac{(e - z)I}{z}, \ z = \frac{eI/K_i}{1 + I/K_i}$$

$$K_s = \frac{(e - y - z)s}{y}, \ y = (e - z)\frac{s/K_s}{1 + s/K_s}$$

and hence

$$y = e\left(1 - \frac{I/K_i}{1 + I/K_i}\right)\left(\frac{1}{1 + K_s/(s)}\right)$$

$$= \frac{e}{(1 + I/K_i)(1 + K_s/s)}$$

and the rate of the reaction, v,

$$= \frac{V}{(1 + I/K_i)(1 + K_s/s)}$$

This can be written in the form

$$1/v = \frac{1}{V}(1 + I/K_i)(1 + K_s/s)$$

so, as with competitive antagonism, the graph of $1/v$ against I is a straight line. The value of K_i can be obtained directly from this graph because, when $1/v$ is zero, $(1 + I/K_i)$ must also be zero (for $1/V$ and $(1 + K_s/s)$ cannot be), hence $I = -K_i$

If the experiment is repeated with a second concentration of substrate, another line passing through the same point should be obtained (Fig. 1.10). For a further discussion of enzymes, see Dixon and Webb (1958)

Conclusion

There is a striking similarity between the interactions of enzymes, substrates, and inhibitors (or heterogeneous catalysts, reactants, and poisons) and tissues, agonists, and antagonists. Although the conditions are not absolutely comparable, information from enzymic reactions may be helpful in discussing drug-receptor mechanisms. Such information will clearly be especially useful when the receptor mechanism is known to involve enzymic processes.

On the whole, an enzymic mechanism for the action of a drug at a receptor would be easier to understand than a physical one. The concept of efficacy fits simply into an enzymic scheme and the biological stimulus may be provided by the accumulation of a reactive substance which enters into the next stage of a series of reactions. A physical mechanism, such as the alteration of a particular part of a membrane, is more difficult to understand. It would seem less likely to lead to a variable efficacy, because the change in structure brought about by the fit of the molecule to the receptor should either occur or not occur. The question, however, is completely open, and many drugs are known, agonists as well as antagonists, whose action at the receptor cannot involve their destruction or modification; it is difficult to see how these can be acting directly on an enzyme system.

In this book an attempt is made to discuss the relationships between the chemical structures of drugs and their effects at the most fundamental level, e.g. on enzymes. It will be seen, however, that at present it is not often possible to do this, or even to form an idea of the enzymes involved.

This approach necessitates a new consideration of drugs. These are not regarded according to their usefulness or according to the method of synthesis (to name two common ways of looking at them), they are considered only for their effects in one particular situation.

Classification of Sites Where Drugs May Act

This account of a chemical approach to pharmacology, therefore, necessitates a definition of the sites at which such an approach may be possible. Ideally these should be simple in structure and in the mechanism by which the effects are produced, and also such that they can be isolated from the rest of the body, so that the effects of drugs on them may be studied directly. Sites are accordingly discussed in the following order

1 Peripheral nerve fibres
2 The junctions of nerve-fibres with muscle, or of nerve fibres with nerve fibres (as in ganglia), and
3 Muscles or organs

No attempt will be made to discuss the actions of drugs on the central nervous system Although much progress has been made in understanding the processes occurring in the central nervous system and it is now possible to perform direct experiments, for instance, on single nerve-cells in the spinal cord, it is still difficult, and often impossible, to specify the sites within the central nervous system where drugs are acting It is therefore questionable whether it is justifiable to discuss the actions of drugs on the central nervous system in chemical terms It is possible, for example, to correlate chemical structure with analgesic activity (ability to alleviate pain), and very interesting relationships have been observed (reviews by Eddy, 1959, Beckett and Casy, 1962), but little is known about the nature of pain, what pathways in the central nervous are associated with it, and whether the observed effects of the drugs are all produced by the same mechanism For these reasons, and for others set out in the preface, this discussion is limited to the actions of drugs on certain peripheral sites

Before proceeding to a discussion of the relationships between chemical structure and pharmacological activity, however, it is important to know how the activity is assessed The next chapter deals with the principles on which quantitative pharmacology is based At this point, too, the reader who is unfamiliar with anatomy, physiology, and biochemistry should turn to the Appendix This is designed to provide him with sufficient background to make the rest of the book intelligible

II

Principles of Quantitative Experimental Methods in Pharmacology

Importance of quantitative experiments – Biological variation – Normal distribution – The accuracy of a mean – Comparison of two means – Types of response – Graded responses – All-or none responses – Disguised all or none responses – Comparisons of the activity of different drugs – Activity of antagonist drugs – Inhibition of enzymes – Differences between observed activity and fundamental activity – Conclusion

Importance of Quantitative Experiments

The growth of pharmacology in this century has to a large extent been due to the development of quantitative methods for the estimation and comparison of drugs This has come about largely by the application of statistics to the interpretation of biological results The correct assessment of the meaning of the results of pharmacological experiments is so important that a brief account is included here so that the chemist may realize some of the difficulties involved and may judge critically and intelligently the value of results quoted in later chapters and to be found in the literature

Biological Variation

When a chemistry student performs a titration he expects to obtain results which are consistent to a high degree Any serious variation in the answer arises from errors by the observer, not from the experiment itself In bio logical experiments, however, the situation is far more complex Great variations in response may be found even when all the obvious variables, strain and species of animal, sex, time of the year, temperature and so on, have been controlled An example of this is given in Fig II 1, which shows the variation in the body temperature among a group of medical students taken at the same time and in the same environment This biological variation, as it is called, should be one of pure chance and can be treated as a problem in mathematical probability

Chemists should be familiar with a somewhat similar variation, that of speed among molecules The chances are greatest that a molecule will have a speed of about the root mean square velocity, but some will have speeds much less and some much more, than this The chances of very high speeds, however, are less than those of very low speeds and consequently the distribution is skew (Fig II 2) In biological problems there may be no reason why extremely low values should be more likely than extremely high ones and the distribution may be symmetrical about the mean This situation may also be familiar to some chemists – such as microanalysts – in whose experiments
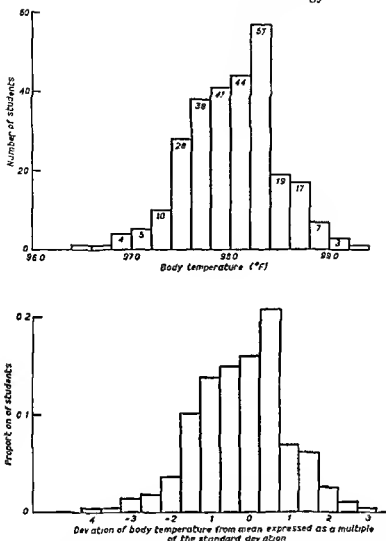
FIG II 1 *Distribution of body temperature among medical students attending a lecture*

There were 276 students and the mean body temperature was 98 1° F The upper histogram shows the number of students whose body temperature lay in the range (0 2°) indicated by the blocks, the lower histogram shows the same results but with the number of students expressed as a proportion of the total and the temperature expressed in terms of the mean and the standard deviation, which in these experiments was 0 4° F the range 98 2°–98 4°, for example, becomes +0 25 to +0 75σ (Drawn from results of Du Bois, 1948)

errors of observation are likely to be significant In reading a micro-burette, or in performing a combustion analysis, the limits of accuracy may be set by the ability of the observer to read his instruments consistently The chances are (or ought to be) that he will record something approaching the true value, but there may be a significant chance that he will record some value

slightly less or slightly greater His results will therefore be subject to biological variation arising from the observer In biological experiments this variation arising from errors of observation is usually much less important than the variation arising from the biological experimental material In order to assess the value of pharmacological results it is particularly important to have some understanding of how these results are likely to be affected by biological variation
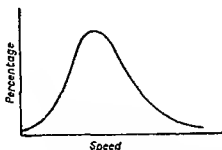
FIG 11 2   *Distribution of speed among molecules*

Normal Distribution

Figure II 3 shows the variation in the dose of digitalis which kills a cat This dose, the individual lethal dose for a particular cat, was obtained by infusing digitalis slowly until just enough had been given to stop the heart Most of the animals in this experiment were killed by a dose somewhere near the mean lethal dose ($\bar{x}$ or LD$_{50}$), but there is a considerable scatter due to more sensitive and less sensitive animals in the group A measure of this scatter can be made by calculating the variance, V, being obtained by summing the squares of the deviation of each result, x, from the mean, x, and dividing by the number of degrees of freedom (one less than the number of animals, n)

$$V = \frac{\Sigma(x - \bar{x})^2}{n - 1}$$

The actual value of $x - \bar{x}$ may be positive or negative, depending on whether x is greater or less than $\bar{x}$ and $\Sigma(x - \bar{x})$ should be zero The value $(x - \bar{x})^2$, however, can only be positive and provides a sensitive indication of the scatter because the squaring process gives great weight to divergent results It might seem logical to divide this total by the number of results, rather than $n - 1$, but with $n - 1$ deviations from a mean value, the nth deviation follows as a matter of course (because $\Sigma(x - \bar{x}) = 0$)

Another term which may be used to measure scatter is the standard deviation, σ, which is the square root of the variance

$$\sigma = \sqrt{V} = \sqrt{\frac{(\Sigma x - \bar{x})^2}{n - 1}}$$

A large standard deviation indicates a wide scatter of results and a flat distribution curve, a small standard deviation indicates that the results lie close together
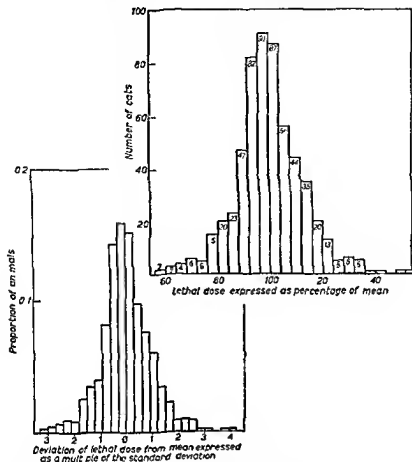
FIG II 3   *Variation in the dose of digitalis which kills a cat*
*There were 573 cats, and the doses were expressed as a percentage of the mean
lethal dose The upper figure shows the number of cats for which the lethal dose lay
in the range (4 per cent of the mean) indicated by the blocks for example, for 82
animals the dose was between 92 and 96 per cent of the mean The lower figure shows
the same results but with the number of cats expressed as a proportion of the total
and with the dose range expressed in terms of the standard deviation, which in
these results was 13 03 per cent of the mean, for example, the range 92–96 per cent
of the mean corresponds to −0 614 to −0 307 σ (Drawn from results of Van
Wijngaarden, 1926)*

If the distribution is now plotted, not in terms of the actual dose, x, but
in terms of the mean dose and the standard deviation, σ (i e against N,
where $x = \bar{x} + N\sigma$), all curves should assume the same shape, that of a
normal frequency curve The probability of obtaining results lying within the
range $\bar{x} + N_1\sigma$ and $\bar{x} + N_2\sigma$, where $\bar{x}$ is the mean, σ the standard deviation,
and N_1 and N_2 are variables, can be calculated purely from mathematical
considerations of probability and is given by the expression

$$P = \int_{N_1}^{N_2} \left(\frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}N^2} \right) dN$$

This integral is not a simple one, but values of P for particular values of N_1 and N_2 can be obtained from tables. For example, when $N_1 = -1$ and $N_2 = +1$, $P = 68.2$ per cent, i.e. this proportion of the results should lie within one standard deviation on either side of the mean. For $N_1 = -2$ and $N_2 = +2$, $P = 95.5$ per cent, and this proportion of the results should lie within two standard deviations of the mean. The experimental results in
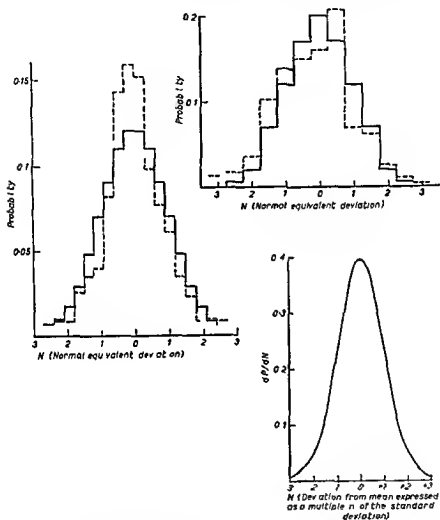
FIG II 4   *Calculated and observed probability distributions*
   The top section shows the probability of obtaining results in the experiment with medical students lying in the range indicated (the width of the blocks is 0.5 σ) calculated from the expression $\int_{N_1}^{N_2}\left(\frac{1}{\sqrt{2\pi}}\,e^{-\frac{1}{2}N^2}\right)dN$, *compared with the experimental results (Fig II 1, shown as the broken line)*
   The middle section shows the same for the results in the experiments with cats (the width of the blocks is 0.3 σ and the results in Fig II 3 are shown as the broken line)
   The bottom graph shows the function $\frac{1}{\sqrt{2\pi}}\,e^{-\frac{1}{2}N^2}$, *i.e. dP/dN, plotted against N*

Fig II 1 agree fairly well with this, 61 7 per cent of the students had a body temperature within the range 97 7–98 5° F (the mean was 98 1° F and the standard deviation 0 4° F) and 91 3 per cent within the range 97 3–98 9° F. In the experiments with cats the percentages were 73 3 within $\pm\sigma$, and 93 9 within $\pm 2\sigma$ It should in fact, be possible to calculate the values of P for the limits used in producing the histograms shown in Fig II 1 and Fig II 3 and to compare these with the proportion of results observed to be lying in that range (Fig II 4)

The Accuracy of a Mean

The normal distribution of results applies not only to single determinations distributed about a mean but to mean values themselves determined experimentally When a group of experiments is repeated it does not follow that the same mean value will be obtained as previously (though the new mean should be much closer to the previous mean than should one single result to another) The experimentally determined means should be distributed normally about the true mean (obtained only from an infinite number of experiments) and an estimate of the standard deviation of this distribution, which is called the standard error, ϵ, is given by

$$\epsilon = \frac{\sigma}{\sqrt{n}} = \sqrt{\frac{\Sigma(x-\bar{x})}{n(n-1)}}$$

If this standard error were the true standard deviation of the experimental means about the true mean, the chances are 68 2 in 100 that the true mean will lie within the range $\bar{x} \pm \epsilon$, and 95 5 in 100 that it lies within $\bar{x} \pm 2\epsilon$ As it is only an estimated value the range must be extended and a correction factor introduced The corrected value is called the t-deviate and will depend upon the number of degrees of freedom involved in estimating the standard error The larger the number of experiments, the more likely the standard error is to approach the true value of the standard deviation of the means about the true mean Tables of the t deviate indicate the range within which the true mean is likely to lie at a particular level of probability For example, with 5 degrees of freedom (6 experiments) it is 2 57 at a probability of 0 05, e there are only 5 chances in 100 that the true mean lies outside the range $\bar{x} \pm 2 57\epsilon$ (compare with 1 96 for an infinite number of degrees of freedom) The actual values $\bar{x} - 2 57\epsilon$ and $\bar{x} + 2 57\epsilon$ are referred to as the fiducial limits at a probability of 0 05

Mean values quoted in the literature should therefore be given either with their standard error or with the number of experiments on which they are based, or with a statement of the fiducial limits at a particular level of probability

Comparison of Two Means

It is often necessary to decide whether the differences between two mean values, $\bar{x}_1$ and $\bar{x}_2$, are real or could simply have arisen from biological variation From the standard error, ϵ_1 and number of degrees of freedom ($n_1 - 1$)

for the first set of results, it should be possible to express the chances of obtaining the value $\bar{x}_2$, the mean of the second set of results Likewise it should be possible to express the chances of obtaining $\bar{x}_1$ in the second set of experiments with a mean of $\bar{x}_2$, a standard error of ϵ_2 and $n_2 - 1$ degrees of freedom By combining these considerations it should be possible to express the chances that the two means will be separated by a particular distance expressed as some multiple of a function derived from the two standard errors If they are further apart than this, the means are significantly different at that particular level of probability the test for significance is in fact,

$$\bar{x}_1 - \bar{x}_2 > t\sqrt{\epsilon_1^2 + \epsilon_2^2},$$

where t is the t deviate based on the total number of degrees of freedom, $n_1 - 1 + n_2 - 1$, and the level of probability chosen For example, the t deviate at a probability of 5 in 100 and 8 degrees of freedom is 2 31 If therefore $\bar{x}_1 - \bar{x}_2 > 2\ 31\ \sqrt{\epsilon_1^2 + \epsilon_2^2}$, where the total number of experiments, $n_1 + n_2 = 10$, there are less than 5 chances in 100 of obtaining the two means $\bar{x}_1$ and $\bar{x}_2$ (by chance) and they can be termed significantly different at a probability of 0 05 It may, of course, be argued that if there are as many as 5 chances in 100 of the two means being obtained (by chance), they are not significantly different, and it rests with the investigator to decide what chances will be convincingly significant (to himself and to other workers) in any particular set of conditions

Types of Response

In biological experiments two types of response are possible graded and all-or-one ('quantal')

A graded response is one whose size varies (over a range) depending upon the effect of a drug For example, the contraction of a piece of muscle in response to the action of a drug may vary from no contraction at all up to the maximal contraction of which the muscle is capable Biological variation will be observed in that the response obtained with a particular dose of drug will not be the same on one piece of muscle as it is on another, nor, even, will exactly the same response be obtained when the effect of a particular dose is tested repeatedly on the same piece of muscle

In a toxicity experiment, however, only two responses are possible, life or death, hence the name all or none Biological variation will here be observed in that the dose which kills one animal may not kill another The quantitative treatment of these two types of response is necessarily different, although the principles underlying the statistical treatment of the results are essentially the same

Graded Responses

In experiments where the action of a drug gives a graded response, it should be possible to compare the effects produced by a standard solution and an unknown solution by plotting the size of the response against the dose and comparing the two graphs so obtained If the standard and unknown contain

the same drug (in different concentrations) the two graphs should be parallel and an estimate of the concentration in the unknown could be made The graph of dose against response, however, is usually a sigmoid curve, and it may be extremely difficult to decide how this line should actually be drawn A plot of the logarithm of the dose, rather than the dose itself, against the response, on the other hand, is more likely (see p 6) to be a straight line, at least over the middle part of the graph The log dose-response relationship, therefore, is more suitable for quantitative work The accurate placing of two points on the standard graph and two points on the unknown should suffice to fix the two lines (called 'regression lines') accurately, provided the graphs really are linear This assumption forms the basis of the 'four point assay'
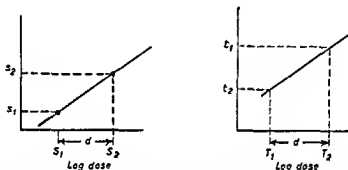
FIG II 5   *Four point assay mean responses, s_1 and s_2 are shown to doses S_1 and S_2 of standard and t_1 and t_2 to T_1 and T_2 of unknown The actual units of the doses of standard and unknown may be quite different for example, moles with the standard and grammes of powder with the unknown This does not matter, because the logarithm of the dose is being used and the interval d, on the log scale is the same for both In this particular experiment the amount of unknown used is producing a bigger response than the amount of standard*

By preliminary testing two doses of unknown T_1 and T_2 and two doses of standard, S_1 and S_2 are found which produce between about 25 per cent and 75 per cent of the maximal response The calculations are simplified if the ratios of the doses T_2/T_1 and S_2/S_1 are the same it is often 2, but the actual value will depend upon the steepness of the dose-response curve, sometimes doubling the dose which gives a 25 per cent response will produce an effect greater than a 75 per cent response These doses are then given in a random order a number of times (usually four times each)

Suppose that the mean response to S_1 is s_1, to S_2 is s_2, and so on (Fig II 5) If the regression lines are parallel, the slope, b, will be given by

$$b = \frac{s_2 - s_1 + t_2 - t_1}{2d}$$

where d (the 'log dose interval') is the logarithm of T_2/T_1 (or S_2/S_1) This value is based on information from eight pairs of responses The quantitative

difference between the responses produced by the unknown and those produced by the standard (the 'preparation difference') will be given by

$$\frac{t_2 - s_2 + t_1 - s_1}{2},$$

this value, too, being based on eight pairs of responses. The strength of the unknown (its 'potency') can then be calculated, for the response $\frac{s_2 - s_1 + t_2 - t_1}{2}$ is produced by multiplying a dose by 10^d. Hence the response $\frac{t_2 - s_2 + t_1 - s_1}{2}$ is produced by multiplying the concentration in the standard by 10^M, where $M = \frac{t_2 - s_2 + t_1 - s_1}{s_2 - s_1 + t_2 - t_1} \times d$.

Note. When drugs are given in solution, the dose depends upon both the volume and the concentration. If the volumes of the doses T_1 and S_1 are the same, the potency, 10^M indicates the concentration of drug in the unknown. If, however, V_t/ml of unknown solution are used as T_1 and V_s/ml of standard as S_1, then the concentration of drug in the unknown solution will be

$$10^M \times \frac{V_s}{V_t}$$

The advantage of this type of procedure over plotting the graphs over a range of doses is that it readily lends itself to the statistical assessment of the errors attached to the assay. It is possible, for example, to attach fiducial limits to an answer obtained in this way (see Burn, Finney, and Goodwin, 1952).

All-or-none Responses

In experiments where the response is all or none, the comparison of a standard with an unknown involves the use of groups of animals. Although a dose will either kill one particular animal or fail to kill it, it need not necessarily kill all the animals in a group or fail to kill any. Because of the biological variation of the individual animals, a dose may kill only a certain percentage of the group. If, therefore, several groups of animals are given different doses of the drug, the percentage mortality within a group should vary with the dose.

The percentage mortality can be regarded as a graded response, just as was the contraction of a muscle, considered above, but the graph of dose against percentage mortality is sigmoid, being, in fact, an integrated normal frequency curve. A dose of the drug will kill not only those animals for which it is the individual lethal dose but also all those whose individual lethal dose is smaller than this. This property of the curve can be made use of in order to derive a function of the effect (percentage mortality) which varies linearly with the dose.

Instead of using the expression for probability,

$$P = \int_{N_1}^{N_2} \left(\frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}N^2} \right) dN$$

between the two limits N_1 and N_2, the lower limit, N_1, is taken as $-\infty$, and consequently the value of P will now indicate the chances of obtaining an animal whose individual lethal dose is less than, or equal to, the upper limit, N_2 It should, therefore, correspond to the percentage mortality observed when a dose, $\bar{x} + N_2\sigma$ (σ being the standard deviation), is given to a group of animals As the normal frequency curve is symmetrical, this form of the curve will also be symmetrical, and the mean lethal dose will kill 50 per cent of the animals and is called the LD_{50} Table II 1 shows the values of P for

TABLE II 1
Conversion of Percentage into Probability Units (Probits)

	0	1	2	3	4	5	6	7	8	9
0	—	2 674	2 946	3 119	3 249	3 355	3 445	3 524	3 595	3 659
10	3 718	3 773	3 825	3 874	3 920	3 964	4 006	4 046	4 086	4 122
20	4 158	4 194	4 228	4 261	4 294	4 326	4 357	4 387	4 417	4 447
30	4 476	4 504	4 532	4 560	4 587	4 615	4 642	4 668	4 695	4 721
40	4 747	4 773	4 798	4 824	4 849	4 874	4 900	4 925	4 950	4 975
50	5 000	5 025	5 050	5 075	5 100	5 126	5 151	5 176	5 202	5 227
60	5 253	5 279	5 305	5 332	5 358	5 385	5 413	5 440	5 468	5 496
70	5 524	5 553	5 583	5 613	5 643	5 674	5 706	5 739	5 772	5 806
80	5 842	5 878	5 915	5 954	5 994	6·036	6 080	6 126	6 175	6 227

	0 0	0 1	0 2	0 3	0 4	0 5	0 6	0 7	0 8	0 9
90	6 282	6 287	6 293	6 299	6 305	6 311	6·317	6·323	6 329	6 335
91	6 341	6 347	6 353	6 359	6 366	6 372	6 379	6 385	6·392	6·398
92	6 405	6 412	6 419	6 426	6 433	6 440	6 447	6·454	6·461	6 468
93	6 476	6 483	6 491	6 498	6 506	6 514	6·522	6·530	6 538	6 546
94	6 555	6 563	6 572	6 580	6 589	6 598	6 607	6 616	6 626	6 635
95	6 645	6 655	6·665	6 675	6·685	6 695	6·706	6 717	6 728	6 739
96	6 751	6 762	6 774	6 787	6 799	6 812	6 825	6 838	6·852	6 866
97	6 881	6 896	6·911	6·927	6 943	6 960	6 977	6 995	7 014	7 033
98	7 054	7 075	7 097	7 120	7 144	7 170	7 197	7 226	7 257	7 290
99	7 326	7 366	7 409	7 457	7 512	7 575	7 652	7 748	7 878	8 090

Percentages 0–80 in units of ten on top, units of one across the top, 90–99 9, in units of one on left in units of 0 1 across top of lower section of table

Bliss, (1938)

the expression integrated from $-\infty$ up to N, i e the percentage of a group likely to be killed by a dose which is distant from the LD_{50} by the value $N\sigma$ In this Table the value N, the normal equivalent deviation, is replaced by $N + 5$, this value is called a probit (probability unit) and is often more convenient to use than the normal equivalent deviation because it is positive for all percentages likely to be met

The graphs of percentage mortality against dose vary greatly from one drug to another, although all are sigmoid in shape Some drugs produce a

steep curve, others a much flatter one If a large number of groups of animals were used in a toxicity experiment and if the standard deviation (of the individual lethal doses about the mean, $\bar{x}$) were known, it should be possible to reproduce the integrated normal frequency curve itself (Fig II 6) Instead of plotting the percentage mortality against the dose, x, it would be plotted against the normal equivalent deviation, N, where $N = \dfrac{x - \bar{x}}{\sigma}$ Drugs with a flat percentage–mortality–dose curve will have a large value for σ, those with a steep curve will have a small value, and the plot of percentage mortality
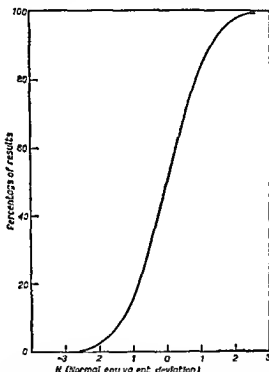
FIG II 6 *Integrated form of normal frequency distribution (cf Fig II 4) percentage of results likely to be equal to or less than a value above or below the mean by a particular multiple, N, of the standard deviation*

against N will reduce them all to the standard theoretical shape The determination of the percentage mortality in a group of animals produced by a dose of a drug is a very simple procedure compared with the lengthy process of determining the individual lethal doses of the same number of animals It might seem relatively easier, therefore, to verify the integrated normal frequency curve as compared with the difficulty of verifying the normal frequency curve itself The value of the standard deviation, however, can only be obtained by determining the individual lethal doses of a number of animals, so the reproduction of even the integrated normal frequency curve is not a simple matter

If it is assumed that the individual lethal doses are distributed normally,

then the graph of $\frac{x - \bar{x}}{\sigma}$ against the percentage mortality should fit the integrated normal frequency curve (Fig II 7) The value $\frac{x - \bar{x}}{\sigma}$ would actually equal N, the normal equivalent deviation corresponding (on the theoretical curve) to the particular percentage mortality The values $\bar{x}$ and σ are constant, hence the graph of x against N should be a straight line whose equation is $x = \bar{x} + N\sigma$ (Fig II 8)

The graph of dose against the normal equivalent deviation (or the probit) of the percentage mortality accordingly forms the basis for quantitative
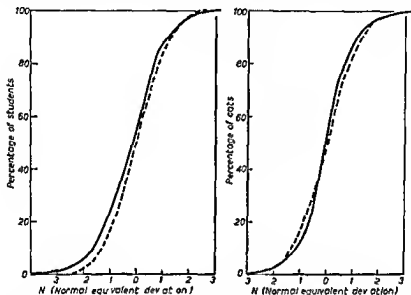
FIG II 7   Integrated forms of Fig II 1 (left) and II 3 (right) percentage of the total number of the group whose body temperature or lethal dose was less than, or equal to a value above or below the mean by a particular multiple, N, of the standard deviation The dotted curve is the integrated form of the theoretical frequency curve

experiments with drugs which produce all-or none responses Toxicity tests in only two groups of animals should be sufficient to establish the LD_{50} ($\bar{x}$) of a drug (and also σ, if required) and comparison of a standard and an unknown can be carried out by a technique such as the four point assay described for graded responses Statistical procedures can be used to describe the fiducial limits of these estimates

In this account it has been assumed that individual lethal doses are distributed normally, and the results in Fig II 4 and Fig II 7 can be taken to support this Gaddum (1945), however, has pointed out that in many instances it is the logarithm of the dose, rather than the dose itself, which appears to have a normal frequency distribution The procedures described here, therefore, may need to be modified by using the logarithm of the dose rather than the dose itself The method of Litchfield and Wilcoxon (1949)
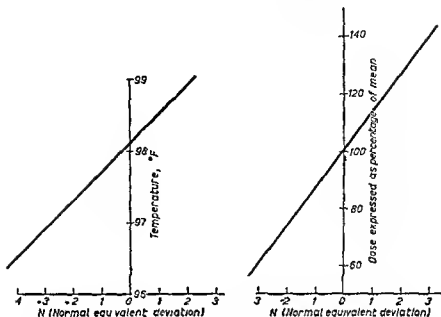
FIG II 8 *Graph of x against N, on the left are the results of Fig II 1 and on the right those of Fig II 3 In both these experiments σ was known (0 4° F in the former and 13 03 per cent of the mean dose in the latter), and so the graph must necessarily be a straight line In a toxicity experiment x would not be determined but the dose, x, would be plotted against the value of N corresponding with the percentage mortality observed as found from the standard curve (Fig II 6) The result will be a straight line only if the variation really fits the normal frequency curve*

for the evaluation of LD_{50}, the slope of the line and the confidence limits, using nomograms and probability graph paper instead of logarithms and tables, is based on this assumption of a 'log normal' distribution

Disguised All-or-None Responses

Many biological structures are composed of individual units which give all-or-none responses, although the whole structure appears to give a graded response The phrenic nerve-diaphragm preparation of the rat (Bülbring, 1946) is an example It consists of a strip of muscle, the diaphragm, attached to a lever and stimulated electrically (usually about ten times per minute) by electrodes placed on the phrenic nerve (Fig II 9) If a drug such as (+) tubo-curarine chloride is added to the bath in which the preparation is kept, the contractions gradually diminish in size, the percentage reduction after a standard time can be used as an indication of the blocking effect produced If this percentage reduction is regarded as a typical graded response, it would be reasonable to expect the graph of the logarithm of the dose against the percentage reduction to be a straight line and to use this graph as a basis for assays (Chou, 1947)

In fact, however, both the nerve and the muscle are made of fibres, each of which either function fully or not at all The drug may therefore be

regarded as producing an all-or-none effect, fundamentally the same as in a toxicity experiment The percentage reduction in the contractions of the muscle should be a measure of the percentage of the muscle-fibres 'knocked out' (The way in which they are blocked is immaterial, the drug actually blocks the junction of the nerve-fibres and the muscle fibres) It would be expected therefore that the graph of the dose against the probit of the percentage reduction of the contractions would be a straight line, but in fact the graph of the logarithm of the dose against the probit of the percentage reduction appears to be more nearly linear (Barlow and Ing, 1948) The errors involved in this kind of experiment, however, make it difficult to attach great significance to the apparent fit of the results to a particular
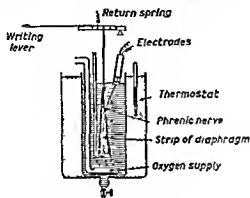
FIG 11 9 *Rat diaphragm preparation.*

mathematical expression Because of the movement of the lever and its inertia, the actual size of the tracing is not an accurate measure of the biological response Further, the population of muscle fibres may not be homogeneous, a few in particular may be contributing much more to the response than the others All the same, it is important to recognize a disguised all-or-none response for what it is improvements in recording technique may well justify in future plotting the probit of the percentage effect, rather than the percentage effect itself, against the dose (or logarithm of the dose) if assays are to be performed as accurately as possible (see, for example, Bevan, 1960)

Comparison of the Activity of Different Drugs

The quantitative methods outlined above show how it may be possible, given a standard solution of a drug, to estimate by bioassay the concentration in an unknown solution Such bioassays are extremely important for the standardization of biologically active materials which have not been isolated completely pure, or even for substances like insulin, which have been obtained crystalline, but are not normally prepared commercially in such a high degree of purity They may also be extremely useful for the assay of highly active substances, such as adrenaline, in concentrations which are too low to be estimated satisfactorily by chemical methods

The comparison of the biological activity of one drug with that of another,

however, is a different matter In a bioassay it is assumed that the regression lines for the standard and the unknown are parallel, as indeed they should be when the same drug is present in both solutions When two different drugs are being tested, however, there is no reason why this should be so

Further, when two solutions of the same drug are being compared, the rate of onset of the effect, the duration of the effect and its intensity all depend only on the amount of drug administered The pharmacological test may actually only measure one of these parameters The three are not necessarily connected, however, and the time course of the effects produced by two different drugs may be quite different If they are, it is intrinsically impossible to express numerically (as a 'potency') the activity of one drug in terms of another It is impossible, for example, to compare numerically the activity of a drug which acts rapidly but for a short time with that of a drug which acts slowly for a long time A potency figure can, of course, be obtained if the two are compared by a pharmacological test which only measures one parameter of the effect, say rate of onset, or duration Such figures are, however, completely meaningless, as they will depend entirely on the para meter being measured

The numerical comparison of the activity of two drugs depends upon establishing that the time courses of their effects are comparable and that their dose-effect curves are parallel If these conditions hold, the relative activity of the two can be expressed by comparing the amounts (or concentrations) which produce comparable effects The result can be expressed as a potency, or, more suitably, as an equipotent molar ratio, that is the number of molecules of the test drug which produce the same effect as 1 molecule of the standard It is important to remember that a high potency indicates high activity but a high equipotent molar ratio indicates low activity

Two drugs may produce the same biological response (e g death, con traction of a piece of muscle) by actions at completely different sites or by different mechanisms even if they act at the same site If their time courses are comparable and their dose-effect curves parallel it is likely, but does not necessarily follow, that they are acting at the same site and in the same way If their time courses are not comparable and their dose-effect curves are not parallel this may be taken as an indication that they are not acting in the same way, but again this does not necessarily follow, because the differences may arise from other factors, such as rates of transport or penetration, and not from differences in the fundamental mode of action It may be desirable, therefore, to attempt to obtain some idea of the relative activity of two drugs even when their dose-effect curves are not parallel In these circumstances it is necessary to plot the curves and to express the potency (or equipotent molar ratio) as a range, specifying the level of effect at which the comparisons are made This range may be considerable, e g in Fig II 10 the potency of β-Eucaine relative to cocaine is between 0 56 and 0 22 depending on the level of anaesthesia at which the comparison is made Sometimes the figures can do no more than indicate an order of magnitude but in certain circum stances even this information may be of some use
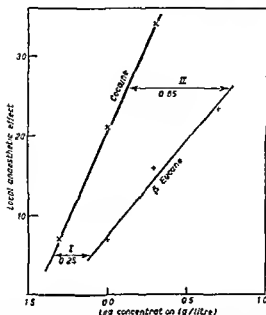
FIG II 10   *Comparison of the local anaesthetic effects in guinea pigs of cocaine and β-Eucaine, at the level I, comparable effects would be produced by 1 8 times as much β-Eucaine as cocaine, but at level II 4 5 times as much would be needed (Re drawn from results of Bülbring and Wajda, 1945)*

Activity of Antagonist Drugs

The biological responses so far considered have all been positive in the sense that the compounds produce an effect which can be measured, even if the effect itself is negative, such as a slowing in heart rate Many substances, however, are of pharmacological interest not because they themselves produce effects but because they antagonize the effects of another drug, it is necessary to be able to express this antagonistic activity quantitatively Sometimes the antagonism results in a biological response which is positive – for example, the action of atropine in antagonizing acetylcholine results in a dilatation of the pupil of the eye – but usually the only effect of the antagonist is to reduce the effect of the agonist so that more must be given in order to produce the same biological response The change in the size of the response to a standard dose of agonist could be used as the basis of a bioassay of standard and unknown solutions of an antagonist A development of this type of assay for comparing two different antagonists, however, is unsatisfactory as it would depend upon graphs of dose against antagonistic effects being parallel at all concentrations of the agonist

If the antagonism is competitive and obeys the Law of Mass Action it should be possible to express absolutely the antagonistic activity of a particular drug in relation to a particular agonist The comparison of the activity of two antagonists could then be made by studying each of them separately as antagonists of the same agonist

The measurement of absolute antagonistic activity depends greatly upon the accurate measurement of the antagonistic effect The change in the actual size of the response to the agonist is not a suitable measure of this because the dose-response curves for different preparations are not necessarily parallel A 50 per cent reduction in the response to the agonist will indicate a much smaller antagonistic effect when the preparation has a steep dose-response curve than when it has a flat one It is, therefore, more accurate to try to keep the biological response at a constant level (and on the steep part in the middle of the dose–response curve) The antagonistic effect is assessed by finding out by how much the concentration of agonist must be multiplied in order to do this This figure has been called the dose ratio (Gaddum, Hameed, Hathway, and Stephens, 1955), but it is only a measure of the antagonistic effect produced, not of the absolute antagonistic activity Higher concentrations of the antagonist will produce a bigger dose ratio The theoretical background to this situation has already been discussed on page 12 If the antagonism is competitive, the dose ratio $A/a = 1 + BK_B$ where A is the concentration of the agonist which, in the presence of a concentration B of antagonist, produces the same response as a concentration a of agonist alone When A/a is fixed, the value of B will depend only on its absolute antagonistic activity, K_B Clark and Raventos (1937) used the logarithm of the concentration of antagonist which produced a dose ratio of 10 as a measure of antagonistic activity (a large negative value indicating high activity, see Table I 1)

Schild (1947) uses the logarithm of the reciprocal of the concentration (a positive quantity) as the index of activity and calls this pA_x, where x is the dose ratio This value should be constant for a particular pair of agonists and antagonists, at a particular value of x, at a particular temperature, and for a particular preparation

This is, however, always assuming conditions of equilibrium, and it is therefore necessary to indicate how far this is likely to be true by specifying the time that the tissue and the antagonist have been left in contact

For a dose ratio of 2, $BK_B = 1$ or $K_B = 1/B$ and the value of pA_2 is log K_B (page 13) Other values of pA are related to this, for example, when $A/a = 10$, $BK_B = 9$, hence log $K_B = 0.95 + pA_{10}$ (Schild, 1947, 1957)

When antagonism is competitive, estimates of pA_x in different laboratories have been found to agree remarkably well (Reuse, 1948, Gaddum, 1957, Arunlakshana and Schild, 1959)

The equation $A/a = 1 + BK_B$ can also be written $\dfrac{A - a}{B} = aK_B$, consequently, for a constant response, the ratio $\dfrac{A - a}{B}$ should be constant When a is much smaller than A, the ratio becomes A/B, and this is the 'drug ratio' used to describe antagonistic activity by Gaddum, Hameed, Hathway, and Stephens (1955) A maximal response of the preparation is obtained with the agonist and then the antagonist is added in a concentration which produces a considerable effect (a dose ratio of more than 5) The

concentration of agonist which produces a 50 per cent response in the presence of this concentration of antagonist is then found The drug ratio is obtained by dividing this concentration of agonist by the concentration of antagonist It should remain constant over a fairly wide range of concentrations Although it does not have as much absolute significance as pA_x, or K_B, it provides a convenient way of expressing roughly the number of molecules of antagonist which block the effect of one molecule of agonist (provided the concentrations are expressed as molarities which is not always done)

Both pA_x and the drug ratio only have absolute significance when the antagonism is competitive One of the simplest methods of testing for competition is to compare pA_2 and pA_{10} If the antagonism is competitive, $A/a = 1 + BK_B$, $pA_2 = \log K_B$, $pA_{10} = \log K_B - 0.95$, hence $pA_2 - pA_{10} = 0.95$ (Schild, 1947, Marshall, 1955, Ariens and Van Rossum, 1957, Schild, 1957, Arunlakshana and Schild, 1959)

Another method is to plot the dose ratio minus one, $A/a - 1$, against the concentration of antagonist If the antagonism is competitive, this should give a straight line (Fig II 11) To cover a wider range of concentrations, Schild (1960) has plotted the logarithms of these values
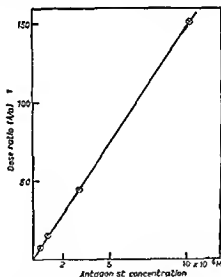
FIG 11 11   Competitive antagonism linear relationship between dose-ratio $(A/a) - 1$ and antagonist concentration The substance, 5 5 diphenyl 2 oxopentyltriethyl ammonium, $Ph_5CHCH_2CH_2COCH_2NEt_3$, was antagonizing the effects of acetyl choline on the isolated guinea pig ileum (Scott, 1962)

In certain circumstances pA_x values may be obtained with antagonists which are not competitive, and the dose-response curve for the agonist in the presence of the antagonist may not be parallel with the curve when no agonist is present To overcome the criticism of the value of pA_x on the grounds that the antagonist may alter the slope of the dose-response curve of the agonist (Guarino and Bovet, 1949), Schild (1949) suggested that pA should always be

determined with concentrations of agonist which produce 50 per cent of the maximal response of the tissue It becomes necessary to see what the value of $pA_2 - pA_{10}$ is likely to be in these circumstances

The equation $y = \dfrac{(A)K_A}{1 + (A)K_A}\left(\dfrac{1}{1 + (B)K_B}\right)$ on page 15

can be written

$$y + (A)K_A y + (B)K_B y + (A)K_A(B)K_B y = (A)K_A$$

$$(A)K_A = \frac{y(1 - (B)K_B)}{1 - y(1 + (B)K_B)}$$

When no antagonist is present,

$$(a)K_A = \frac{y}{1 - y}$$

So n, the dose ratio $= [A]/(a) = \dfrac{1 + (B)K_B}{1 - y(1 + (B)K_B)}\ (1 - y)$

$$n - ny - n(B)K_B y = 1 + (B)K_B - y - (B)K_B y$$

$$(B)K_B = \frac{n - 1 + y - ny}{1 - y + ny} = \frac{(1 - y)(n - 1)}{1 + y(n - 1)}$$

$$1/(B) = K_B \frac{(1 + y(n - 1))}{(1 - y)(n - 1)}$$

and

$$pA_n = \log K_B + \log \frac{1 + y(n - 1)}{(1 - y)(n - 1)}$$

$$pA_2 - pA_{10} = \log\left(\frac{1 + y}{1 - y}\right) - \log\left(\frac{1 + 9y}{(1 - y)9}\right)$$

$$= \log\left(\frac{9(1 + y)}{1 + 9y}\right)$$

This value therefore varies with y, for example, when $y = 0\,5$,

$$pA_2 - pA_{10} = \log\frac{9 \times 1\,5}{5\,5} = 0\,39,$$

but as y approaches zero, $pA_2 - pA_{10}$ approaches log 9, the value for competitive antagonism The value of using $pA_2 - pA_{10}$ as a test for competitive antagonism therefore depends very much on the circumstances, in particular on the confidence which can be placed in the experimental results

Inhibition of Enzymes

The pharmacological properties of some drugs depend, in part at least, on their ability to block certain enzymes, and it may be necessary to demonstrate this action on isolated enzyme systems This is purely a biochemical problem and the inhibitory activity can be expressed by the inhibitor constant (see page 22) In practice the pharmacologist often merely compares the rate of the reaction in the presence of a concentration of the drug with the rate in its absence and expresses the effect as a percentage inhibition of the control rate The concentration of antagonist which reduces the rate to half that of

This is usually followed by conjugation with glucuronic acid, an amino-acid, or sulphate The conjugate is a highly polar compound with a low pK_a which fails to penetrate membranes, and as such is filtered in the glomerulus of the kidney but not reabsorbed from the tubule, and so is likely to be excreted This picture is over simplified (for reviews see Albert, 1960, Brodie, 1956, Brodie and Hogben, 1957, Brodie, Gillette, and La Du, 1958, Williams 1959), but may be of some help is assessing the value of estimates of activity obtained from experiments in whole animals

Even in experiments with isolated pieces of tissue mounted in a bath containing a suitable salt solution, factors such as diffusion and ability to penetrate membranes may greatly affect the results Ing and Wright (1931), for instance, showed that estimates of the neuromuscular blocking activity of quaternary ammonium salts (p 97) on the isolated frog gastrocnemius muscle were valueless because they depended on the rates of diffusion of the drugs into the site of action, i e on the ionic weight, and not on the true biological activity of the compounds To obtain a proper estimate of the activity of the drugs, the preparation had to be perfused and the drugs added to the perfusion fluid so that they had rapid access to the site of action

Conclusion

The correct interpretation of quantitative results in pharmacology, without which it is impossible to discuss relationships between structure and activity, depends upon an appreciation of the assumptions involved in the calculation of the results and of the factors which may mask the fundamental activity of a drug

III

Actions on Peripheral Nerve-Fibres

The Nerve-Cell and Nervous Conduction

Like any other cell, the nerve-cell consists essentially of solid matter, partly in suspension in water (e g proteins) and partly in solution (e g ions), enclosed by a membrane This membrane can be considered (review by Danielli, 1958) as being composed of a layer of lipoid 2 molecules thick, the molecules being arranged tail to tail with the hydrophilic groups outside, covered with a layer of protein (Fig III 1) the membrane is approximately
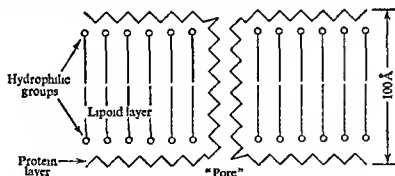
FIG III 1   *Membrane of nerve cell (after Danielli, 1958)*

100 Å thick, but in medullated nerves is covered, except at the nodes, by a very thick myelin sheath (a Schwann cell Fig Appendix II 8)

The axon membrane, as distinct from the myelin sheath, cannot be regarded as a completely continuous lipoid-barrier Although certain substances can pass into the cell by a process apparently of diffusion, water and certain small molecules (e g methanol) and ions can penetrate the membrane extremely rapidly The membrane can therefore be described as semipermeable in the sense that the word is used in chemistry in the discussion of osmosis, but it has additional properties which are difficult to explain in chemical terms In particular it is selectively permeable to potassium ions and

49

apparently impermeable to sodium ions In a normal, resting, nerve-cell the concentration of potassium ions inside the cell is greater than that outside, whereas the concentrations of sodium ions and chloride ions inside are less than those outside It is supposed (review by Hodgkin, 1958) that this situation is brought about by the extrusion of sodium ions from the inside of the cell by some mechanism called the 'sodium pump', which is capable of operating against a considerable chemical potential gradient In conditions where sodium ions are being extruded from the cell, the rate of extrusion is greatly reduced by 2 4-dinitrophenol, but returns to the original value when the 2 4 dinitrophenol is removed This substance is known to interfere with metabolic processes which yield energy and it is thought that the energy for driving the sodium pump is derived from adenosine-triphosphate

The cell membrane is polarized, the outside being positive with respect to the inside It is possible to insert an electrode inside some cells, such as the giant axon of the squid, and to measure the potential difference between the inside and the outside This potential can be considered to arise from the differences between the internal and external concentrations of K^+, and to a lesser extent Na^+ and Cl^- Considering K^+ alone, the resting potential will be given by

$$V = \frac{RT}{F} \ln \frac{K^+_o}{K^+_i}$$

where K^+_o is the concentration outside the cell and K^+_i the concentration inside For the squid axon the ratio $\frac{K^+_o}{K^+_i}$ is $\frac{20}{400}$ and, if R is taken as 8 3 Joules/degree, F as 96,500 coulombs, and T as 290° absolute,

$$V = \frac{8\ 3 \times 290 \times 2\ 3}{96,500} \times (-1\ 3)$$

$$= -75\,mV$$

This value is actually too high and allowance must be made for a contribution from the differences between the internal and external concentrations of Na^+ (inside outside = 50/440) which will set up a potential difference of the opposite sign The equation for the resting potential can be written

$$V = \frac{RT}{F} \ln \frac{(K^+_o) + b(Na^+_o)}{(K^+_i) + b(Na^+_i)}$$

and for the squid axon the value of b, the ability of Na^+ to penetrate the membrane compared with that of K^+, is roughly 0 01

If an electric potential is applied in opposition to the resting potential, thus depolarizing the membrane, the permeability is completely altered Sodium ions, instead of being preferentially excluded from the cell, now pass into it The concentration of Na^+ inside the cell increases and the membrane adjacent to the point of application of the potential becomes positively polarized on the inside This polarization, in its turn, alters the permeability of the membrane and so on The process, once started, is self-perpetuating

and spreads along the axon in both directions away from the point of application of the stimulus The rate at which it spreads depends on the properties of the membrane The increased permeability to Na^+, however, is only transitory and the original ionic *status quo* is quickly restored

During stimulation of an axon, electrodes placed outside and inside it record a negative potential difference between the surface and the inside, followed by an ultimate return to the positive resting potential (see Fig Appendix II 15) Although the movements of Na^+ ions contribute most to the electrical changes observed, they do not account completely for the size and shape of this action potential If, however, the movements of other ions are studied, K^+, Cl^- and, in some types of axon, organic anions such as isethionate ($HOCH_2CH_2SO_3^-$), it is possible to account virtually completely for the electrical changes which occur during nerve stimulation Hodgkin (1958) has calculated that during stimulation of squid nerve, approximately 20,000 ions are in movement per square micron of membrane

The movements of ions through the membrane, both in the resting condition and during the various stages of the passage of an action potential, may be brought about by either or both of two mechanisms, by the opening of pores or by the action of enzymes It may even be that in some conditions the pore and the enzyme are one and the same thing It must not be supposed, however, that the membrane becomes extensively porous when it is depolarized Calculations based on the heat associated with the passage of an impulse indicate that this would be produced by as little as 1 per cent change in the surface area of the membrane The movements of ions during the passage of an impulse (e g the entry of sodium ions), moreover, cannot be adequately accounted for by simple diffusion, but must be regarded as a 'facilitated diffusion' (Danielli, 1958)

The membrane appears to contain, therefore, mechanisms for the active transport of particular ions, and one of the great puzzles is to understand how it distinguishes between sodium ions and potassium ions It has been suggested that it contains pores of such size that the potassium ions can pass through, whereas the more hydrated, and therefore larger, sodium ions cannot Values of the degree of hydration of various ions obtained by a number of methods (Table III 1, Bell, 1958) do not support this idea, but there are definite physical differences between Na^+ ions and K^+ ions The K^+ ion has a much higher conductance in water than the Na^+ ion (64 reciprocal ohms at 18° C as against 43 reciprocal ohms), and whether or not this indicates that it is less hydrated, a difference of this order of magnitude might well account for the differences in ability to penetrate membranes Glasses have been discovered which can distinguish between sodium ions and potassium ions (Eisenman, Rudin, and Casby, 1957, Hinke, 1959, 1961) and can be used as electrodes for estimating the activities of sodium ions or potassium ions in solution It is, therefore, possible to suppose that the differential transport of sodium ions and potassium ions has a physical basis, but the possibility that the mechanism is entirely enzymic cannot be dismissed, although there is no indication what enzymes might be involved

TABLE III 1

Hydration of Ions

Method	Li+	Na+	K+	Mg++	Ca++	F	Cl-	Br-	I-
(i)	5	4	4	t2	t0	—	4	2	1
(ii)	3	t	1	—	—	0	1	1	1
(iii)	3	2	1	5	4	2	1	1	1
(iv)	4	5	5	t2	t0	4	2	1	1

Figures show the average number of water molecules associated with the ion as deter mined from

(i) Ionic mobilities
(ii) Diffusion experiments
(iii) Activity coefficients
(iv) Compressibility experiments

— Indicates not known

Bell (1958)

Possible Effects of Drugs on Nervous Conduction

There are only a limited number of effects which a drug can produce on a nerve impulse It may lower the threshold at which the nerve responds to stimulation, it may even, itself, disturb the nerve sufficiently to give rise to an impulse and it may block the transmission of an impulse It does not appear that a drug can alter the rate of conduction of a nerve impulse along a nerve fibre, but some compounds, such as the veratrine alkaloids, can cause repetitive firing, i e when one stimulus is applied the response consists of a volley of impulses instead of a single one Stimulation of nervous tissue in any of these ways, however, is not common The majority of substances which are of interest because of their effects on nerve-fibres, block the conduction of impulses and can be called peripheral nervous depressant drugs

Uses of Peripheral Nervous Depressant Drugs

The most obvious use of drugs which block conduction in nerve fibres is to abolish sensation to enable operations to be performed These may be quite simple, such as the sewing up of a wound or the lancing of an abscess, or more complex, such as the anaesthesia of nerves of the jaw in dentistry, of the throat, of the eye, or even of the spinal cord itself Peripheral nervous depressant drugs are also used quite widely in the alleviation of pain, such as that arising from an exposed wound, or, together with antihistamine drugs (page 355), they may be used for treating irritating conditions of the skin

Information Sought in Local Anaesthetic Tests

The effectiveness of a drug depends very greatly on the conditions in which it is used To be any help, therefore, in assessing the possible therapeutic

value of a drug, tests must be made in conditions resembling the clinical situation, even though these may not reveal fundamental information about the properties of the drug This is true even of the testing of peripheral nervous depressant drugs In theory it should be perfectly simple to test all such drugs at the most fundamental level, i e for their ability to block the passage of an action potential along single nerve fibres In practice this is not often done (see below) and might not even be particularly helpful from a practical point of view For instance, this test would not distinguish between substances which can penetrate mucous surfaces and those which cannot, and the latter would be useless for operations on the throat The testing of peripheral nervous depressant drugs (and of all other drugs) has therefore usually been made in circumstances which reveal, as much as possible, the potential usefulness of the drug in man and it is important to examine how far this reveals (or does not reveal) the fundamental activity of the compound

So far it has always been found that sensory fibres are more sensitive to the action of drugs which block conduction than are motor fibres Peripheral nervous depressant drugs, therefore, produce loss of sensation in lower concentrations than those causing paralysis and so are called local anaesthetics It is not clear whether it is justifiable to assume that all drugs block sensory fibres before motor fibres, and many pharmacological tests of local anaesthetics involve reflex arcs, i e both sensory and motor fibres, and could not distinguish between a block of either component It is also important to recognize that drugs acting at the synapse, the junction of sensory and motor fibres in the spinal cord, may appear to be active in such tests

Preparations Used

Single Fibres

The most fundamental information is likely to be obtained by tests on single nerve fibres from which the myelin sheath has been removed The fibre is stimulated electrically and the drug is tested for its ability to suppress the action potential The particular type of nerve chosen depends largely on the ease with which it can be dissected out and kept alive Skou (1954), for instance, used single de-sheathed fibres from the sciatic nerve of the frog

Nerve Trunks

Bennet, Wagner, and McIntyre (1942) tested local anaesthetics for their ability to reduce the spike of the action potential of the isolated frog's sciatic nerve, and Truant (1958) and Åstrom and Persson (1961) have used a similar preparation The disadvantage of using nerve trunks of this type, however, is that they contain mixed types of fibre, sensory and motor, medullated and non medullated Douglas and Ritchie (1960) have described a preparation of the saphenous nerve of the cat in which the non medullated sensory fibres (Type 'C' on page 443) can be followed for some distance from their origin in the skin Drugs can be injected into the saphenous artery so that they reach the area served by the nerve The method was designed to test the effects of acetylcholine on sensory endings, but has been adapted by Zaimis (1961)

for the testing of local anaesthetics Action potentials are recorded from the saphenous nerve in response to mechanical tapping of an appropriate area of skin and drugs can be tested for their ability to suppress the action-potential

Reflex Arcs

In the frog's sciatic plexus preparation (Sollmann, 1918, Bülbring and Wajda, 1945) the frog is decapitated and eviscerated It is then suspended by the front legs so that the hind legs hang free If one of these is immersed in a solution of hydrochloric acid, impulses travelling along a spinal reflex arc result in the contraction of the limb away from the acid The drug solution is placed in the abdominal cavity and tested for its ability to block the reflex

In the rabbit's or guinea-pig's cornea test (Sollmann, 1918) the animal is made to blink by touching the cornea with a fine bristle or wisp of cotton-wool The drug solution is applied to the cornea and tested for its ability to block the blink reflex

In the goldfish or tadpole test (Baum, 1899, Adams *et al*, 1926, Meyer, 1937) the fish are placed in a solution of the drug in a thermostat and tested for the disappearance either of spontaneous activity or of the reflex response to a stimulus, such as the disturbance of the bath by a glass rod

In the guinea pig or human weal test (Chance and Lobstein, 1944, Bülbring and Wajda, 1945, Mongar, 1955) the twitch response of the skin to a pin-prick is used A small amount of the drug is injected intradermally (between the dermis and the epidermis, i e just under the outer layer of the skin) raising a weal The drug is tested for its ability to abolish the twitch response when the weal is pricked lightly

In Mongar's modification of the method, the drug is tested on an open blister, produced by the application of cantharidin to the skin Results obtained with this method are much more consistent than with the intra-dermal weal and fewer estimations are needed to obtain results with comparable fiducial limits

Other preparations which have been used include the reaction to the placing of a clip on a mouse's tail (Bianchi, 1956)

Assessment of Activity

To compare the activity of different drugs it should only be necessary to determine the concentrations which produce identical effects Difficulties arise, however, in measuring the effect Blockage of conduction is an all or-none response so it is impossible to assess quantitatively the intensity of the block. Skou (1954), for example, found the concentration of drug which, in conditions of equilibrium, just blocked conduction (he checked that with slightly lower concentrations conduction was not blocked) The attainment of equilibrium, however, requires a long time, and in other tests equilibrium conditions are not achieved In these the effect noted may be the rate of onset of block or the duration of block, but not usually both If the drugs being tested can, in suitable concentrations, produce identical effects (i e which

have the same time course) their activities can be assessed by comparing these concentrations. It does not follow, however, that the drugs may be able to do this, in which case a true comparison is impossible (page 41), it will not be apparent in most tests whether they do or not

In the frog's sciatic plexus, rabbit cornea, and goldfish tests the time when the reflex disappears is taken as the index of activity and concentrations of drugs producing block in the same time are compared. The graph of the time of onset of block against the logarithm of the concentration of drug is approximately a straight line. If the slope of this line for the test drug is the same as that for a standard drug, it is possible to express the activity of the test in terms of the standard by a simple assay procedure (page 34)

In the intradermal weal test an attempt is made to combine measurement of the duration of the effect with an assessment of the intensity of the effect during the recovery stage. Five minutes after the injection the weal (usually outlined in ink, as it may subside during the period of the experiment) is pricked lightly six times. As a control, the skin well away from the weal is also tested. Unanaesthetized skin will twitch each time it is pricked, fully anaesthetized skin will fail to do so. When anaesthesia begins to wear off some of the population of sensory endings in the weal may have recovered while others are still blocked, consequently a number of twitches intermediate between 0 and 6 may be obtained. The weal is tested every 5 minutes until 30 minutes have elapsed since the injection. The total number of negative responses (i.e. times when there was no twitch) is calculated. Within limits the graph of the logarithm of the concentration of drug against the effect is a straight line, and if the lines for two drugs are parallel, an assay can be performed to determine relative potency. Lands and Hoppe (1956) estimated the concentration of a drug producing a score of 5, i.e. threshold effects, and compared these values (which they called TAC 5)

In all tests involving reflex arcs, substances which block motor nerve-endings or the neuromuscular junction (page 90) may appear active. In the frog's sciatic plexus and goldfish tests, substances which block conduction at synapses in the spinal cord may appear active. The goldfish test is, in fact, used for general central depressant drugs (page 380). Furthermore, because the tests measure either the rate of onset of the effect or its duration, potency figures determined by one method alone may give misleading information about substances which act slowly for a long time or rapidly for a short time. Another point to be noted is that the cornea is a mucous surface, and results obtained in this test depend upon ability to penetrate such surfaces so may bear no relation to results in other tests, such as the intradermal weal

The usual pharmacological screening tests for local anaesthetic activity (the cornea and intradermal weal test) indicate the concentration of drug which would be effective in conditions which resemble, as nearly as possible, those in which it might be used. This effectiveness depends on other factors besides the ability to block conduction. One factor, already mentioned, is the ability to penetrate mucous (or other) surfaces. Another important factor is the ease with which the drug may be eliminated from the site where

c

it has been applied It may be possible to increase the effectiveness of a drug by increasing its ability to penetrate surfaces (e g by adding hyaluronidase, an enzyme which hydrolyses hyaluronic acid, a polysaccharide constituent of connective tissue) It may also be possible to increase effectiveness by preventing the elimination of the drug from the site of action by adding a vasoconstrictor substance, usually adrenaline (page 294), to reduce the bloodflow to this area Some local anaesthetics are themselves vasoconstrictor, but the effectiveness of many others is markedly potentiated by the addition of adrenaline Effectiveness may even be increased further by a combination of both these procedures

Most of the estimates of local anaesthetic activity quoted below have been determined by tests which assess effectiveness, and it must be borne in mind that this is not the same thing as fundamental activity The relative activities of a number of the more widely used local anaesthetics are shown in Table II 2

Cocaine and Related Compounds

Wöhler, in 1860, observed that cocaine produced numbness when placed on the tongue, and Anrep (1879) showed that it abolished sensation when injected intradermally It was Koller, however, who, in 1884 at Freud's suggestion, showed its effectiveness on the eye and its usefulness in operations on the eye Within a year it was used in surgery of the throat and in dental practice It is active on mucous surfaces (hence its effects on the tongue and the eye), but is not now used because less toxic substances are available

The general structure of cocaine was worked out by Willstätter (1896), who showed it to be benzoylmethyl ecgonine, but the absolute configuration has recently been determined (Findlay, 1954, Kovács, Fodor, and Weisz,

(−)-Cocaine, III 1

1954, Hardegger and Ott, 1955, review by Fodor, 1960) (−) Cocaine (III 1) is 2R methoxycarbonyl 3S benzoyltropine
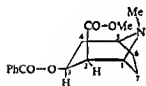
It has 4 asymmetric centres, but those numbered 1 and 5 can be disregarded because the pyrrolidine ring locks them *cis* Ecgonine is a 2 carboxylic acid derived from ψ tropine, in which the hydroxyl group is equatorial Ecgonine and

ψ ecgonine differ in the conformation of this carboxyl group, in ecgonine it is axial and in ψ ecgonine it is equatorial (+)-ψ Cocaine is derived from ψ ecgonine and is 2S methoxycarbonyl 3S benzoyltropine The absolute configuration of (−) cocaine (showing that it is as written above and not the mirror image) was established by showing that the ecgoninic acid to which it was oxidized was identical with the ecgoninic acid synthesized from (+)-S glutamic acid (Fig III 2)

Findlay (1956) has reported the preparation of the two other pairs of isomers of cocaine These must have the tropine configuration (a 3 axial hydroxyl group), and Findlay has suggested that in *allococaine* the 2 carboxyl group is equatorial and in *allopseudococaine* it is axial *Allococaine* has also

been prepared by Zeile and Schulz (1956) but the material they obtained apparently contains half a molecule of water of crystallization and has a much higher melting point (156-8° as against 82-4°)
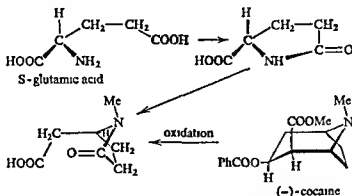
FIG 111 2 *Establishment of the absolute configuration of (−) cocaine*

(−) Cocaine, (±)-cocaine, (+) ψ cocaine, (±)-ψ cocaine, benzoyltropine, and benzoyl-ψ tropine (*Tropacocaine*) have been studied by Gottlieb (1923) His results, obtained with the frog s sciatic plexus and dog's cornea preparations, do not permit a quantitative comparison, but they indicate that all the compounds have about the same order of activity, the relative potency being (+)-ψ-cocaine > (±)-ψ cocaine > (−) cocaine > (±) cocaine on the sciatic plexus and (−)-cocaine > (+)ψ cocaine (±) ψ cocaine > (±) cocaine on the eye Benzoyl ψ tropine appeared to be slightly more active than benzoyl tropine, but these substances were compared separately from the others Whatever the exact potency of the isomers may be, it seems clear that the arrangement of the groups in the cocaine molecule is not critical

Zeile and Schulz (1956) reported that their isomer of cocaine was ineffective when tested on the guinea pig's cornea in a concentration as high as 5 per cent (w/v), although cocaine produced anaesthesia in a concentration of 1 per cent This result is puzzling This isomer, unlike the isomers studied by Gottlieb, should have an axial 3 benzoyl group, as it is related to tropine instead of ψ tropine This difference in structure, however, would not be expected to deprive the compound entirely of activity because benzoyltropine is not greatly different in activity from benzoyl ψ tropine There is the possibility that the compound cannot penetrate mucous surfaces This is known to be

α -cocaine III.2

true of another isomer of cocaine, 'α' cocaine (III 2), in which the methoxycarbonyl and benzoyl groups are attached to the same atom In theory there should be two isomers, corresponding to the tropine and the ψ tropine structures but in practice the synthesis from tropinone cyanhydrin leads only to one isomer, in which the methoxycarbonyl group is axial (Heusner, 1957) This substance was reported by Willstatter (1896) not to be a local anaesthetic because it did not produce numbness when placed on
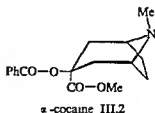
the tongue Foster, Ing, and Varagic (1955), however, found that, though it was inactive on the rabbit s cornea, it was quite potent when tested on the frog's sciatic plexus or in the guinea pig weal, the equipotent molar ratios relative to cocaine were 1 7 and 5 respectively

Among compounds closely related to cocaine, activity is shown by aliphatic esters of benzoylecgonine other than methyl (III 3) and, to some extent, by aromatic esters of methylecgonine (see, for example, Poulssen, 1890, Gray,

R = Me R = $\langle \_\_\_\rangle$-CO R" = Et Pr Bu allyl or benzyl   III 3

R = Me R' = $\langle \_S\_\rangle$-CO R" = Me   III 4

R = H   R = $\langle \_\_\_\rangle$-CO R" = Me   III 5
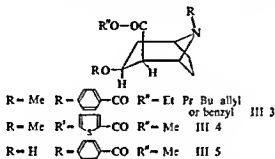
1925) The α thienylester (III 4) is as active as cocaine (Steinkopf and Ohse, 1924) Norcocaine (III 5) is at least as active as cocaine, but cocaine metho-salts ecgonine, methylecgonine, and benzoylecgonine are inactive

Von Braun et al (1918, 1920, 1922) studied the effect of moving the benzoyl ester group to the end of a polymethylene chain attached to the nitrogen atom (in place of the methyl group) The most active of these dihydroecgonidines was the compound with three methylene groups, but the corresponding eegonidine (III 6 Ekkaine) was more active, being comparable with cocaine (test method not specified)

Ekkaine, III 6
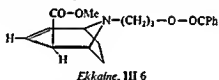
The First Synthetic Compounds

The elucidation of the structure of cocaine was rapidly followed by the discovery of local anaesthetic activity in simpler molecules The first synthetic compounds, α Eucaine (III 7, Vinci, 1896) and β-Eucaine (Benzamine, Beta-

α Eucaine III 7
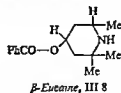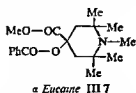
β-Eucaine, III 8

caine, III 8, Vinci, 1897), contain the piperidine ring of cocaine but lack the pyrrolidine ring Both substances produced effects on the guinea pig s cornea, but β Eucaine was more active, though not as active as cocaine α Eucaine

possesses a plane of symmetry, but β-*Eucaine* contains two asymmetric carbon atoms King (1924) succeeded in obtaining both β-*Eucaine* and *iso*-β-*Eucaine* and in resolving each of these The compounds were all approximately equi-active on the rabbit's cornea, but in the frog's sciatic test, the (+)- and (−)-forms of β-*Eucaine* were found to be equiactive and stronger than the (+)- and (−)-forms of *iso*-β-*Eucaine* The absolute configuration of these compounds is not known

Esters of Benzoic Acid

Ehrlich (1890, Ehrlich and Einhorn, 1894) had expressed the view that the benzoic ester group in cocaine was the 'anaesthesiophoric' group, endowing the molecule with activity, and, after Vinci's introduction of the *Eucaines*, many benzoic esters of simple aminoalcohols were found to be local anaesthetics Among the first compounds of this type were *Amylocaine* (*Stovaine*, III 9, Fourneau, 1904) and *Alypin* (*Amydricaine*, III 10, Impens and

$$PhCO-O-\underset{\underset{Et}{|}}{\overset{\overset{Me}{|}}{C}}-CH_2NMe_2$$

Amylocaine, III.9

$$PhCO-O-\underset{\underset{CH_2NMe_2}{|}}{\overset{\overset{CH_2NMe_2}{|}}{C}}-Et$$
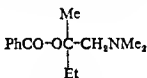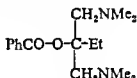
Alypin, III 10

Hofmann, 1905) Other examples are *Piperocaine* (*Metycaine*, *Neothesine*, III 11, McElvain, 1927), the cinnamic ester *Apothesine* (III 12, Sollmann, 1918), and *Hexylcaine* (III 13, Beyer *et al*, 1948) These compounds are not

$$PhCO-O-CH_2CH_2CH_2N\underset{Me}{\bigcirc}$$
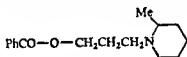
Piperocaine, III 11

$$Ph-CH=CH-CO-O-CH_2CH_2NEt_2$$
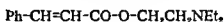
Apothesine, III 12

strikingly active (see Tables III 2 and III 3) although they are sufficiently effective to have been used clinically *Hexylcaine*, its *cyclo*-pentyl analogue and the benzoic ester of 2-*iso*propylamino-*n* butan-1-ol (III 14) were among the most active of a large group of compounds studied by Kuna and Seeler

$$PhCO-O-\underset{Me}{\overset{|}{CH}}-CH_2-\underset{|}{\overset{H}{N}}-\bigcirc$$

Hexylcaine, III 13

$$PhCO-O-CH_2-\underset{\underset{Et}{|}}{\overset{}{CH}}-NH\textit{iso}Pr$$
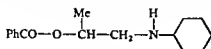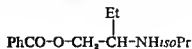
III 14

(1947) On the rabbit's eye these were almost as active as cocaine (equipotent molar ratio, 1 3) and in a test similar to the guinea pig weal they were more active than Procaine (see below), the equipotent molar ratio relative to Procaine being 0 2 to 0 5 and indicating a ratio relative to cocaine of 1 3

TABLE III 2

Local Anaesthetic Effects Equipotent Molar Ratios Relative to (-) Cocaine

		Test								
	Single fibres	Weal					Cornea			Mouse
		Human		Guinea pig			Rabbit	Guinea-pig		
Cinchocaine	0 0019	0 11	0 068	0 33	0 38	—	0 0084	0 01	—	0 078
Amethocaine	0 0039	—	0 083	—	0 59	—	—	—	—	0 20
Tutocaine	—	0 18	—	—	—	—	1 4	—	—	—
Panthesine	—	0 21	—	—	—	—	0 10	—	—	—
Phenocaine	—	0 29	—	—	—	—	0 91	—	—	—
Ravocaine	—	—	0 33	—	0 71	—	—	—	—	—
Butacaine	—	0 45	0 31	—	—	—	0 48	—	—	—
(+) ψ-Cocaine	—	0 77	—	—	—	—	0 77	—	—	—
Amylocaine	—	1 0	—	—	—	—	1 0	—	—	—
(−)-Cocaine	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
Trapacocaine	0 83	1 4	—	—	—	—	5 6	—	—	—
Intracaine	—	—	—	—	—	—	—	2 1	—	—
Lignocaine	—	—	1 4	—	2 3	2 6	—	2 1	4 2	2 6
β-Eucaine	—	2 4	—	—	—	—	3 7	—	—	—
Procaine	1 8	2 3	3 1	10	7 7	36	17	14	6 7	—
Alypin	—	2 6	—	—	—	—	5 0	—	—	—
Apothesine	—	7 7	—	—	—	—	7 1	—	—	—
Reference	S	H	M	B	L	D	H	Bü	D	W

S = Skou (1954)   H = Hirschfelder and Bieter (1932)   M = Monger (1955)   B = Bulbring and Wajda (1945)   L = Lands and Hoppe (1956)   D = Dofek and Vrba (1959)   Bü = Buchi, Lauener, Raga Boniger and Lieberherr (1951)   W = Weidmann and Petersen (1955)

TABLE III 3

Local Anaesthetic Effects

	Equipotent molar ratios relative to (−)-cocaine on	
	Guinea pig sciatic nerve	Guinea pig cornea
Amethocaine	0 11	0 071
Butacaine	0 50	0 25
Hexylcaine	0 83–1 7	1 1
(−)-Cocaine	1 0	1 0
Piperocaine	2 5	1 1
Procaine	14	5 0

Beyer et al (1945)

to 4 0 *Meprylcaine (Oracaine, III 15, Truant, 1958) is a closely related compound which appears to have the same order of activity, when it was tested for its ability to reduce the size of the action potential in the frog's sciatic nerve-trunk, the equipotent molar ratio of the compound relative to Procaine was found to be 0 037

$$PhCO-O-CH_2-\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{C}}-NHnPr$$
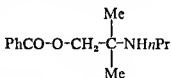
Meprylcaine, III 15

Esters of p-aminobenzoic acid

Einhorn (1905) found local anaesthetic activity among alkyl esters of *p*-aminobenzoic acid These are inevitably weak bases, ethyl *p* aminobenzoate (*Benzocaine, Anaesthesin*), for example, is virtually insoluble in water and too weak a base to form stable salts Such substances could only be used in soothing dusting powders and ointments Alkamine esters of *p*-aminobenzoic acid, on the other hand, like alkamine esters of benzoic acid, are strong bases which form stable salts and consequently can conveniently be injected in solution into the areas where they are wanted The first successful compound of this type, Procaine (*Novocaine, III 16*, Einhorn and Uhlfelder, 1909) is still

$$H_2N-\overset{}{\underset{}{\bigcirc}}-CO-O-CH_2CH_2NEt_2$$
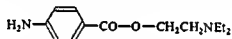
Procaine, III 16

in use, especially as a standard for the comparison of activity in pharmacological tests, although it is not active on mucous surfaces, a disadvantage both clinically and pharmacologically Compounds of this type which have been used clinically include Butacaine (III 17, Volwiler, 1920), *Panthesine*

$$H_2N-\overset{}{\underset{}{\bigcirc}}-CO-O-CH_2CH_2CH_2NnBu_2$$
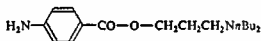
Butacaine III 17

(III 18, Winterstein, 1927, Rothlin, 1929) and *Tutocaine (Butamin, III 19, Schulemann, 1924)

$$H_2N-\overset{}{\underset{}{\bigcirc}}-CO-O-CH_2CH\underset{NEt_2}{\overset{CH_2-\overset{\overset{H}{|}}{\underset{\underset{Me}{|}}{C}}-Me}{}}$$

Panthesine, III 18

$$H_2N-\overset{}{\underset{}{\bigcirc}}-CO-O-\underset{Me}{\overset{Me}{CH}}-CH_2\underset{}{\overset{Me}{CHNMe_2}}$$
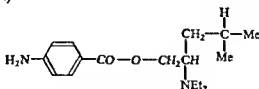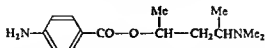
Tutocaine III 19

Local anaesthetic activity in a homologous series usually increases with chain length up to a maximum, beyond which it declines (cf the general depressant activity of *n* aliphatic alcohols, page 380) This type of variation is found, for instance, among alkyl esters of *p* aminobenzoic acid (Adams

et al, 1926 Table III 4) In alkamine esters of *p* aminobenzoic acid an increase
in the length of the chain linking the ester group and the basic nitrogen
increased the basicity but branching of this chain decreased basicity and
increased local anaesthetic activity (Vliet and Adams 1926)

TABLE III 4
Esters of p-Aminobenzoic Acid Tested on Goldfish

$$H_2N-\langle\text{benzene ring}\rangle-COOR$$

R =	Minimal effective concentration mMol./l
Me	0 47
Et	0 13
n Pr	0·04
n-Bu (Butylaminobenzoate)	0 02
n Am	0 022

Adams et al (1926)

The effects of substitution in the side chain amino group are illustrated in
the series of secondary bases studied by Rungk and Epstein (1943) Activity
was maximal when the alkyl group contained between seven and ten carbon
atoms *Octacaine* (III 20) was comparable with Procaine but acted on

Octacaine III.20
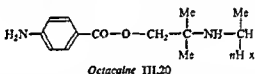
mucous surfaces These compounds appear to have the same order of
activity as the analogous benzoic esters (e g *Meprylcaine*) already discussed

Kuna and Seeler (1947) have tested many alkalmine esters of both benzoic
and *p* aminobenzoic acids and their results do not show much difference
between the members of the two series The activity of the *p* aminobenzoic
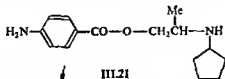
III.21

ester of 2 cyclopropylamino propan 1 ol (III 21) for instance was comparable
with that of *Hexylcaine* and its analogues (see above)

Activity can be increased however by alkylation of the *p* amino group as
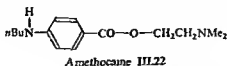
Amethocaine III.22

in Amethocaine (*Butethanol Pantocaine Tetracaine* III 22 Fussganger and
Schaumann 1931) and in either series by substitution of alkoxy groups in
the benzene ring A *n* propyl ether group placed *ortho* to the side chain of

Procaine gives the more active compound *Ravocaine* (*Propoxycaine*, III 23, Luduena and Hoppe, 1952) The position of the ether group does not appear to be critical (Buchi *et al*, 1951) The effects of alkoxy substitution, and also
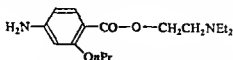
Ravocaine, III.23

of replacing the ester group by amide (see below), are illustrated in Table III 5

The activity of Procaine is also increased by the replacement of the *p* amino group with an ethyl ether group (*Intracaine*, III 24, Rohmann and Scheurle,
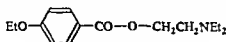
Intracaine, III 24

1936), or by replacing the ester group by thiolester The thiol analogue of Procaine, for example, is more active than Procaine itself (Haosen and Fosdick, 1933) The activity of some alkoxy substituted thiolbenzoates has been studied in detail (Luduena *et al*, 1955, Luduena, 1957, Lands and Hoppe, 1956, Luduena *et al*, 1958) and some of the results are shown in Table III 6 Some of these compounds, e g *Win 4510* (III 25), appear to be more active than any local anaesthetic so far known
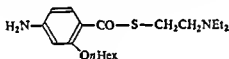
Win 4510, III.25

Amides

The highly active compound *Cinchocaine* (*Dibucaine*, *Nupercaine*, III 26, Lipschitz and Laubender, 1929) contains both an alkoxy group and an amide group The alkoxy group is extremely important for activity and the compound without it is only feebly active The amide group is not so critical, although the β diethylaminopropionic amide of 2 n butyloxy-4 amino-
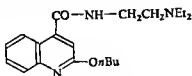
Cinchocaine III.26
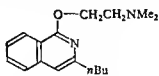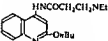
Quotane III.27

quinoline is less active, especially in the cornea test (Table III 5, Buchi *et al*, 1951) Fellows and Macko (1951) have reported high activity in an analogue (*Quotane*, III 27) which lacks the amide group This substance was a little more active than Cinchocaine in the guinea pig weal test and much more active in the rabbit cornea test (the equipotent molar ratio relative to Cinchocaine was about 0 1)

	Equipotent molar ratios relative to (−)-cocaine on	
	Guinea pig weal*	Rabbit cornea
Cinchocaine	0 21	0·010
$HNCOCH_2CH_2NEt_2$ ... O·Bu	0 45	0 089
$HNCH_2CH_2NEt_2$ ... O·Bu	2 5	0 24
Büchi, Ragaz, and Lieberherr (1949) Buchi, Lieberherr, and Ragaz (1951)		
$COOCH_2CH_2NEt_2$	inactive	inactive
$COOCH_2CH_2NEt_2$ ... O·Bu	3 4	6 7
$CONHCH_2CH_2NEt_2$	0 83	1 2
$COOCH_2CH_2NEt_2$ ... nBuO ... O·Bu	1 2	4 0
$CONHCH_2CH_2NEt_2$ ... nBuO ... O·Bu	0 53	0 17
Büchi, Labhart, and Ragaz (1947)		
$COOCH_2CH_2NEt_2$ ... O·Bu, NH_2	0 31	0·050
$CONHCH_2CH_2NEt_2$ ... O·Bu, NH_2	0 31	0 087
$COOCH_2CH_2NEt_2$ ... O·Bu, NH_2 (Sympocaine)	0 31	0 091
$COOCH_2CH_2NEt_2$ ... O·Bu, NH_2	0 31	0 050
Bucht, Stunzi, Flury, Hurt, Labhart, and Ragaz (1951)		

* In the experiments with the guinea pig weal Procaine was used as standard and cocaine was not tested at all, the figures shown here have been calculated assuming that the equi potent molar ratio for Procaine relative to (−)-cocaine in this test is 1 0, this agrees fairly well with the figures shown in Table III 2 and the result for Cinchocaine calculated on this assumption also agrees quite well with the results in Table III 2, the results for *Sympocaine* agree with those shown in Table III 6

TABLE III 6

Local Anaesthetic Properties of Alkoxyphenyl Derivatives

	Equipotent molar ratios relative to (−)-cocaine in the guinea pig weal test
H_2N —⬡— $\overset{O}{\underset{OR}{C}}$—S—$CH_2CH_2NEt_2$	
R = *n*Pr	0 079
= *n* Bu	0 076
= *n*-Pent	0 038
= *n* Hex (*Win 4510*)	0 045
= *iso*-Bu	0 081
H_2N —⬡— $\overset{O}{\underset{OCH_2CH_2Ph}{C}}$—S—$CH_2CH_2CH_2NEt_2$	0 032
H_2N —⬡— $\overset{O}{\underset{OR}{C}}$—O—$CH_2CH_2NEt_2$	
R = *n* Pr (*Ravocaine*)	0 71
= *n*-Bu (*Sympocaine*)	0 27
Cinchocaine	0 38

Lands and Hoppe (1956)

Amides are more stable than esters *in vivo*—Procaine, for example, is destroyed by serum cholinesterase (Kalow, 1952, see page 75)—whereas *Procainamide* (*Pronestyl*, Mark *et al*, 1951) is only slowly destroyed This substance, however, is of interest primarily because of its effects on heart muscle The stability of amides as compared with esters may account, to some extent at least, for the increase in activity (particularly when measured in terms of the duration of action), which results when ester links are replaced by amide (see, for example, Table III 5) One of the most useful local anaesthetics, *Lignocaine* (*Lidocaine, Xylocaine*, III 28,
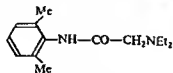
Lignocaine, III 28

Lofgren, 1946) belongs in this class Although it has not the high activity of drugs such as Cinchocaine or Amethocaine, it appears to be clinically extremely safe and reliable Analogues and alkoxy derivatives of Lignocaine have been studied extensively (Buchi *et al*, 1951, Dofek and Vrba, 1959, Åstrom and Persson, 1961, Borovansky, Sekera, and Vrba, 1959)

Phenylurethanes

Some alkamine esters of phenylurethane (e g *Diperodon, Diothane*, III 29, Rider, 1930) have been found to be local anaesthetics The equipotent molar

$$PhNH-CO-O-CH_2$$
$$PhNH-CO-O-CH$$
$$CH_2-N\text{piperidine}$$
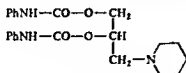
Diothane, III 29

ratio for the most active compounds of this type, γ-diisobutylamino-β-hydroxy-propyl phenylurethane (III 30), relative to cocaine, was about 0 17 in the

$$PhNH-CO-O-CH_2\cdot\overset{OH}{\underset{}{CH}}\cdot CH_2-N\mathit{iso}Bu_2$$

III.30
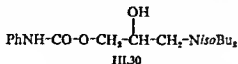
guinea-pig s cornea test and 0 33 in the weal test The α naphthylurethane (III 31, Ramsey and Haag, 1947) had about the same activity

$$NH-CO-O-CH_2\cdot\overset{Me\ \ H}{\underset{Me}{C}}\cdot N-nHept$$

III 31
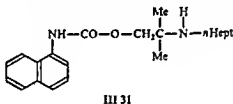
Miscellaneous

It is clear that local anaesthetic activity is not restricted to any particular chemical class of compound *Phenacaine (Holocaine*, III 32), for instance, is a completely different type of structure from any so far discussed The equipotent molar ratio for this compound relative to cocaine in the rabbit's cornea test was about 0 5 (Bonar and Sollmann, 1921)

$$H_3C-C\overset{N}{\underset{N}{\diagdown}}\text{(two p-OMe phenyl groups)}$$

Phenacaine, III 32

$$R-CH-CH-CH_2-N\text{(piperazine)}N-CH_2-CH-CH-R$$
with OH, Me substituents

$$R = n\text{-Hex}, III.33$$
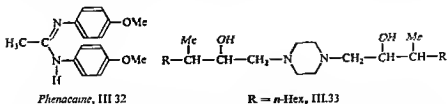
Some piperazine derivatives studied by Fourneau and Samdahl (1930) are particularly active Two series of compounds were studied, in both the activity increased with chain length up to a maximum In the first series the maximum was at the *n* hexyl compound (III 33), for which the equipotent molar ratio relative to cocaine in the rabbit's cornea test was 0 10, in the

second series the n heptyl compound (III 34) was more active than the n hexyl compound and the equipotent molar ratios relative to cocaine were 0 044 and 0 12 respectively

$$R-O-CH_2-CH-CH_2-N \overset{\displaystyle OH}{\underset{\displaystyle}{\big|}} N-CH_2-CH-CH_2-O-R$$

R = n Hept, III 34
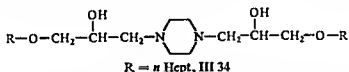
In the even simpler series studied by MacIntosh and Work (1941), activity was maximal in the compound 1-piperidino-2 hydroxytridecane (III 35), for which the equipotent molar ratio relative to cocaine in the guinea pig's cornea test was 0 091 The compound was only about as active as Procaine, however, in the intradermal weal test

III 35
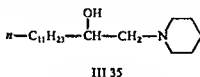
III 36

Some degree of local anaesthetic activity may even be found in compounds which have no basic nitrogen atom Benzyl alcohol produces detectable effects and for saligenin (III 36), the most active of a group of related compounds studied by Maebt (1918), the equipotent molar ratio relative to Procaine was about 5

Because of the finding of local anaesthetic activity in such a variety of molecules it is not surprising that many synthetic compounds, designed for other purposes, have been found also to have local anaesthetic properties Examples are to be found among antimalarial drugs, morphine-like analgesics spasmolytics, antihistamine drugs, quinidine like drugs, and so on In certain circumstances, e g in the testing of drugs using the Trendelenburg preparation (page 144), this possibility should be remembered as it may contribute to the apparent activity of the compound

Quaternary Salts

In spite of the variety of chemical structures which have been found to produce local anaesthesia, one class, quaternary salts, has been regarded as inactive This may partly be because results, discussed below, suggest that quaternary salts ought to be inactive and may also be partly because early work (with cocaine metho-salts, for example, Ehrlich and Emhorn, 1894) indicated that they were inactive

Nador, Herr, Pataki, and Borsy (1953) and Gyermek (1953), however, reported that some quaternary derivatives did have local anaesthetic properties, the activity being greatest in benzylated compounds They used a method described by Herr, Nyiri, and Pataki (1953) in which drugs are tested in rats for their ability to block the response to heat applied to the tail The drug was either injected into the muscles of the tail (theoretically for 'conduction anaesthesia' of the nerve trunks) or into the skin of the tail

(for 'infiltration anaesthesia') Some of the results are shown in Table III 7 The compounds produced effects which lasted for a very long time In the guinea pig cornea test the quaternary compounds were either inactive or much less active than the corresponding tertiary bases The benzyl derivative

TABLE III 7

Activity of Quaternary Compounds, $R_3\overset{+}{N}R$, on the Rat's Tail

		Equipotent molar ratios relative to (−)-cocaine for	
		Infiltration'	Conduction'
$R_3N =$	$R =$		
Procaine		10	71
	Me	11	83
	CH₂Ph	15	30
Cocaine		10	10
	Me	062	48
	CH₂Ph	067	13
Cinchocaine		045	045
	Me	067	24
	CH₂Ph	062	062

Nador, Herr, Pataky, and Borsy (1953)

$R_3N =$	$R' =$		
Benzoyltropine		67	
	n Bu	17	
	CH₂Ph	83	
Benzoyl-ψ tropine (*Tropacocaine*)		43	038*
	n Bu	67	031*
	CH₂Ph	43	050*

Gyermek (1953)

* Relative to Procaine on the frog's sciatic plexus

of Cinchocaine, however, in high concentrations, did produce effects and these lasted for a long time

Hey and Willey (1954) found somewhat similar results among substituted choline phenyl ethers, choline 2 6-xylylether (III 37), for example, produced

effects in the guinea pig weal test comparable with cocaine but lasting between two and ten times as long The action of this compound has been studied in detail and Exley (1957) has shown that it does not block the passage of impulses along the axon but has an effect at the nerve ending In particular it is taken up selectively at postganglionic sympathetic nerve-endings and prevents the release of the transmitter (page 82)

It would seem to be very important, therefore, to test the benzylated
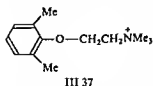
compounds on nerve-fibres The tests with reflexes are not specific enough to be satisfactory, for the reflex could be blocked by many types of drug, including those which blocked transmission at the neuromuscular junction (page 90), a property common among quaternary ammonium compounds

The Active Form of a Local Anaesthetic

With the exception of substances like benzyl alcohol and the quaternary salts considered above, all the compounds which have been discussed are salts of bases To see whether the active form of the local anaesthetic was the ion or the free base, Trevan and Boock (1927) studied the effects of pH on local anaesthetic activity They measured the dissociation constants of a number of local anaesthetics and the smallest concentrations of heavily buffered solutions which produced patchy anaesthesia of the rabbit cornea in 10 minutes The drugs examined were cocaine, Procaine, β-*Eucaine*, *Amylocaine*, and the alkaloid conessine They then plotted the logarithm of the anaesthetic concentration of the drug against the pH of the solution and obtained parallel lines for all the drugs except conessine This is a dibasic substance, and the slope of the line for this drug was twice the slope for the others

If the dissociation is written
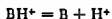
$$BH^+ = B + H^+$$

$$K_a = \frac{[B][H^+]}{[BH^+]}$$

The concentration producing anaesthesia, C, will be $[B] + [BH^+]$,

i e
$$C = [B] + \frac{[B][H^+]}{K_a} = [B]\left(1 + \frac{[H^+]}{K_a}\right)$$

If the base, B, is the active species this relationship would give curves of the type found (Fig III 3)

When $[H^+] \ll K_a$, $C = [B]$ (i e is constant)

In acid solutions, when $\frac{[H^+]}{K_a} \gg 1$,

$$log\, C = log\,[B] + log\,[H^+] - log\, K_a = log\,[B] + pK_a - pH$$

From the dissociation constants and the experimentally determined values of C at pH7, Trevan and Boock calculated the values of C at other values of pH and compared these with the observed values The results do not quite agree and there are three possible explanations for the discrepancy

    1 The difference may be caused by the effect of the change of pH on the tissues This seems unlikely because of the results obtained with conessine and the observation that the activity of benzyl alcohol is independent of pH

    2 The salt itself may have some activity

    3 The pH of the nerve endings affected by the drug may be different from that of the buffer

The effects of pH on local anaesthetic activity have also been studied by Skou (1954), who measured the concentrations of Procaine, cocaine, *Tropacocaine*, Amethocaine, Cinchocaine, and *n*-butanol which just blocked conduction in the de sheathed single sciatic nerve fibre of the frog The experiments were carefully arranged so that the drug should be at the pH of the buffer and also so that equilibrium should be achieved As in the experi-

a = Procaine
b = Amylocaine
c = Cocaine
d = β-Eucaine
e = Conessine
f = Benzylbenzoyl ecgonine
g = Phenylethylbenzoyl
        ecgonine
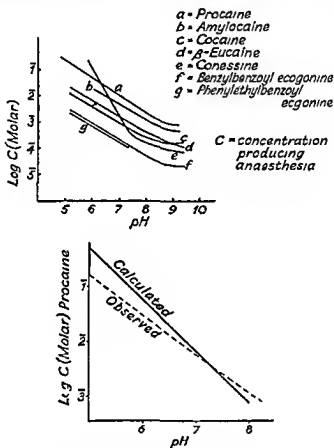
C = concentration
    producing
    anaesthesia

FIG III 3

ments of Trevan and Boock, the concentration of base corresponding to the anaesthetic concentration, *C*, was not absolutely constant, being lower at more acid pH Shanes (1958), however, has pointed out that the calculation should be based on activities and not on concentrations, and if the calculation is made using the activity coefficients obtained in further work (Skou, 1954), the calculated and observed values for *C* at different values of pH become identical (Skou, 1961) These results then indicate that it is the free base which is the effective agent

The Mode of Action of Local Anaesthetics

Local anaesthetic activity is found in a very wide variety of chemical structures and it seems unlikely that such compounds can all be acting on the same biochemical process in the nerve-cell to produce their effects It seems more reasonable to suppose that the action depends either on the physicochemical properties of the compounds or that these physicochemical properties are at least essential, even though the mechanism by which conduction is blocked may depend subsequently on interference with one of a number of biochemical processes

The mode of action of local anaesthetics has been reviewed by Löfgren (1948), Shanes (1958), and Büchi and Perlia (1960) An understanding of how they act must depend upon a knowledge of how they affect the ionic movements across the cell membrane upon which normal transmission of nerve impulses along the axon appears to depend Bennett and Chinburg (1946) found that a variety of local anaesthetics, including cocaine, Procaine, β-Eucaine, Cinchocaine, and Amethocaine, do not affect the resting potential of frog's sciatic nerve-fibres. The blockage of conduction, therefore, does not depend upon depolarization of the axon membrane Bennett and Chinburg, in fact, concluded that the block was the result of the stabilization of certain conditions which must be labile for the conduction of an impulse If, for instance, the drugs stabilized the permeability of the membrane to sodium ions, an action potential could not be propagated

It is not possible, as yet, to say what processes may be stabilized and how this may be brought about Suggestions can, however, be obtained from studies of the physicochemical properties of local anaesthetics Skou (1954) studied the effects of a number of local anaesthetics on single nerve-fibres (see above) and also their distribution between nervous tissue (spinal cord of the ox) and water, their effects on surface (air/water) tension and interfacial (n hexane/water) tension, and their ability to penetrate a monolayer of stearic acid or a monolayer of lipid extracted from frog's sciatic nerve Some of the results are summarized in Table III 8 The relative solubility in nerve-tissue, relative effects on surface and interfacial tension, and relative penetration into a monolayer of stearic acid were estimated by comparing concentrations producing equal effects There appears to be some correlation between solubility in nerve tissue and toxic potency (i e ability to block conduction irreversibly), and between ability to penetrate a monolayer of stearic acid and blocking potency In the experiments with the monolayer of lipid from frog's nerve the drugs were tested in the concentrations which blocked conduction (reversibly) and the degree of penetration into the layer calculated from the change in surface pressure The results indicate that although the minimum blocking concentrations of the drugs vary over 10,000-fold, the equivalent concentration present in a monolayer of lipid from frog's nerve only varies 4-fold

From these results it can be concluded that local anaesthetic activity, for the particular compounds tested, is associated with ability to penetrate a

TABLE III 8

	Minimum blocking concentration*	Relative blocking potency†	Relative toxic potency§	Relative solubility in nervous tissue†	Relative effect on surface tension (surf/water)‡†	Relative effect on interfacial tension (hexane/water)‡	Relative effect on stearic acid and monolayer‖§	Relative effect on mono-layer of lipids from nerve**	Relative concentration in lipid mono-layer††
Procaine	4.6 mM	1	1	1	1	1	1	1	1
Cocaine	2.6	18	31	5	3.5	13	66	13	11
Tropococaine	2.2	21	36	6.6	—	52	19	13	11
Amethocaine	0.01	460	55	48	77	184	253	0.4	0.4
Cinchocaine	0.005	920	188	123	115	766	1,520	0.5	0.6
n-Butanol	68	0.07	0.42	—	—	—	—	1.4	17

Stow (1954)

* pH, 7.0, 22°.
† pH, 7.0, 22°, relative solubility determined using concentrations which block conduction
‡ Relative activity obtained by comparing concentrations whose surface tension is 71.9 dynes-cm, that being the surface tension of the minimum concentration of Procaine which causes block
§ Relative activity obtained by comparing concentrations whose interfacial tension is 43.7 dynes-cm, this being the interfacial tension of the minimum concentration of Procaine which causes block
‖ Relative activity obtained by comparing concentrations for which $F = 10$ dynes-cm.
** Relative activity obtained by comparing the increase in pressure produced by blocking concentrations of the drugs
†† Figures calculated from minimum blocking concentrations and solubility in nervous tissue

(At 22° C the pKa of Procaine is 8.95, of Cocaine 8.70, of Tropococaine 9.32, and of Amethocaine 8.24)

lipoid monolayer It is possible, but not necessarily justifiable, to regard the blockage of conduction as a consequence of this ability to penetrate the lipoid monolayer and to interpret the block by supposing that the substances expand the protein layer on the membrane surface and alter the characteristics of the pores (Shanes, 1958, Skou, 1961) It is also possible to imagine the compounds blocking the pores themselves (Buchi and Perlia, 1960) The value of these pictures depends absolutely on the importance of pores in the transport of ions Until transport mechanisms are better understood, the mode of action of local anaesthetics must remain obscure It is possible, for instance, that the substances require certain physicochemical properties to enable them to penetrate into the membrane but, once in, they may not all be equi-effective or even act in the same say

Straub (1956), from experiments with Procaine on myelinated frog nerve-fibres, suggested that local anaesthetic effects were produced by an action on the sodium ion transport system Shanes and Berman (1955), from experiments with cocaine on de-sheathed toad's sciatic nerve-trunks, concluded that local anaesthetics act only on the outermost layer of the membrane and reduce the permeability to both sodium ions and potassium ions Condouris (1961), from experiments with cocaine on a similar preparation, concluded that cocaine and sodium ions are competitive antagonists So far, then, the results point to only one action by local anaesthetics, on the sodium ion transport systems Changes in the potassium ion transport may be linked with this as there is evidence that sodium ion transport and potassium ion transport may be interdependent (Ussing, 1954)

If these transport systems are enzymic it would be expected that local anaesthetics could be shown to be inhibitors It is very difficult to set about demonstrating this when there is no indication of what types of enzyme might be involved Watts (1949) showed that some local anaesthetics depressed the respiration of brain tissue, in particular inhibiting the metabolism of succinate, but many local anaesthetics are central depressants and these results cannot be very relevant to what may occur in the axon membrane Ryman and Walsh (1955) have shown that some local anaesthetics (in 10^{-3} M concentration) block the synthesis of citrate in the citric acid cycle, but most of the work on the effects of local anaesthetics on enzymes has been with cholinesterases, and to a lesser extent, with amineoxidases At one time it was thought that acetylcholine might be involved in the passage of the action-potential along the nerve axon and local anaesthetics might inhibit its destruction by acetylcholinesterase Skou (1956) did not find any correlation between blocking activity on single de-sheathed nerve-fibres and ability to inhibit the destruction of acetylcholine by acetylcholinesterase or by intact red cells (which contain acetylcholinesterase)

The mode of action of the quaternary derivatives of local anaesthetics (page 67) presents a problem First, it must be clearly established that the substances do in fact block conduction in nerve fibres and not at the sensory endings If this can be demonstrated (and it does not appear to have been done), then information must be obtained about the effect of these substances

on lipoid films It is conceivable that sufficient fat solubility in the benzyl or other part of the molecule might lead to their distribution along the outer surface of the axon-membrane, but this should lead to depolarization of the surface Such an action has been found for the Procaine cation (Straub, 1956) and also for the quaternary methyl derivative of Procaine (Skou, 1961) The effects of the ionised form in increasing the flux of sodium ions might produce a block by a mechanism quite different from that of the unionized base

Although much evidence has been obtained which indicates that local anaesthetics are active in the unionized form and which suggests that quaternary ammonium salts ought not to be acting in the same way, as they are permanent cations, the matter cannot be regarded as being definitely settled The unionized form of a local anaesthetic is clearly important for the transport of the drug to the site of action, but it is still possible that the ion is active at this site Ritchie and Greengard (1961) have performed experiments in which de-sheathed fibres of the cervical vagus nerve of the rabbit (these are mainly C-fibres) were exposed to high concentrations of drugs such as Amethocaine and Cinchocaine The drug solution was then removed and the fibres were bathed in buffer solutions which did not contain the drug The Amethocaine or Cinchocaine present in the fibres took several hours to diffuse out and during this period the effects of pH on the degree of block could be studied If a suitable concentration of drug had originally been selected, it was possible to obtain a situation in which transmission was blocked at pH 7 2, but was markedly and rapidly restored at pH 9 6 The block returned when the buffer was changed back to 7 2, and this process of reducing the block in alkaline pH and restoring it at more acid pH could be repeated several times This was not so easy to demonstrate with Procaine, *however, as this drug appeared to diffuse from the nerve fibres much more rapidly than Amethocaine or Cinchocaine* The transmission in control, untreated, nerve-fibres was little affected by the changes in pH, and the results were taken to indicate that the block of transmission depends upon the presence of cations of the local anaesthetic in the fibre or fibre-membrane

These findings are rather difficult to reconcile with those obtained by Trevan and Boock (1927) and by Skou (1954) and discussed on pages 69 and 70 Ritchie and Greengard (1961) considered that the results of Trevan and Boock indicated the effects of pH on the rate of action of the drugs, i e on their rate of penetration through the cornea, rather than on their absolute activity, but this should not be true for the results obtained by Skou with de-sheathed frog sciatic nerve-fibres in conditions which should be those of equilibrium Possibly the discrepancy may be due to differences in the experimental conditions In Skou's experiments the results were obtained at pH 6 0, 6 5, 7 0, 7 35, and 8 0, whereas in those of Ritchie and Greengard the results were obtained at pH 7 2 and 9 6, so the overlap is small Moreover, in the latter experiments no calcium ions were present, because these would be precipitated in these alkaline conditions Although it cannot be concluded that the ion is not active once it is transported into the membrane, it is very doubtful whether it is essential for the action of local anaesthetics Apart

from the likelihood that this might depolarize the membrane, there are, after all, substances such as benzyl alcohol and saligenin, which have some ability to block conduction and yet are not ionized

Another problem is the blocking action of calcium ions The calcium ion is a normal constituent of body fluids (in concentrations of the order of 5 mM), but an increase in the calcium ion concentration markedly raises the threshold of nerve to stimulation, whereas a decrease increases the excitability It has been suggested that the action of calcium ions, like the action postulated for local anaesthetics, may depend upon an action on the expanded protein film at the cell surface (Skou, 1954, Shanes, 1958) Calcium ions are known to have the effect of stabilizing surface films, for example, of fatty acids (Langmuir and Schaffer, 1936), but the exact mechanism by which they affect nerve fibres is not clear

Relationships Between Chemical Structure and the Usefulness of Local Anaesthetics

The usefulness of local anaesthetics is largely determined by factors other than their absolute potency, e g by their ability to penetrate to a particular site and by their ability to remain at the site Relationships between structure and ability to penetrate mucous membranes, such as the eye, have not been worked out and it is difficult to see how this can be done with the present information available Many substances are active in the weal test but inactive in the cornea test (see, for example, Table III 2) But there are also substances which have high activity in the cornea test, e g the substituted anilides (III 38, 39, and 40, Weidmann and Petersen, 1955), for which the equipotent molar ratios relative to cocaine on the mouse cornea are 0 066, 0 024, and 0 026 respectively, but which are virtually inactive when tested on the mouse tail It is not really possible to deduce anything about ability to penetrate mucous membranes by comparing local anaesthetic activity on the cornea with activity in the weal or other test Such information can only satisfactorily be obtained by a direct study of penetration (for instance, with isotopically labelled drugs)
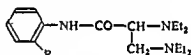
R = COEt III 38
   = COOEt III 39
   = COOnPr, III 40

Grieg, Holland, and Lindvig (1950), however, have found that drugs which anaesthetize mucous surfaces inhibit erythrocyte acetylcholinesterase (page 241), whereas other local anaesthetics do not Addition of eserine (which inhibits the enzyme) enables them to anaesthetize the eye It is difficult to see why this should be so Resistance to the action of butyrylcholinesterases (serum cholinesterases) should prevent the destruction of local anaesthetics and consequently might greatly enhance activity by prolonging the action of the drug This might explain the observations of Kalow and Maykut (1956), who found that in a series of local anaesthetics (alkoxybenzoates and alkoxy-thiolbenzoates) activity was associated with ability to block butyrylcholinesterases which are also inhibited by eserine, page 261 Unless mucous

membranes are exceptionally rich in butyrylcholinesterases, however, it seems unlikely that they can destroy the local anaesthetic so fast that they entirely prevent its action The enzyme could, moreover, only destroy local anaesthetics which were esters, amides or other derivatives should be effective even though they might not have any affinity for the enzyme

In the weal test activity will be greatly increased if the substance produces vasoconstriction or if a vasoconstrictor substance is given along with the

TABLE III 9

Spinal Anaesthesia in Rabbits

	Equipotent molar ratios relative to (−)-cocaine
Symocaine	0 043
Cinchocaine	0 066
Amethocaine	0 12
Ravocaine	0 13
Lignocaine	0 50
(−)-cocaine	1 0
Piperocaine	1 1
Procaine	1 2

$$H_2N-\bigcirc\!\!-\!\!\overset{\overset{\displaystyle O}{\|}}{C}-S-CH_2CH_2NEt_2$$
OR

OR = n Pr	0 0081
= n Bu	0 0037
= n Hex (Win 4510)	0 0023

$$H_2N-\bigcirc\!\!-\!\!\overset{\overset{\displaystyle O}{\|}}{C}-O-CH_2CH_2NEt_2$$
OnHex

	0 021

Compare these results with those shown in Tables III.2 and III 6.

Luduena (1957) Luduena Hoppe, and Borland (1958)

drug The relationships between the structure and vasoconstrictor activity of local anaesthetics have not been worked out It might be expected that a resemblance to sympathomimetic amines (page 294) should lead to activity Cocaine produces vasoconstriction by potentiating the action of the sympathetic transmitter substances (page 340) The stability of the drug to butyrylcholinesterases in serum and to amineoxidases inside the cells are other factors, not studied systematically, which would lead to increased usefulness

One further point about the weal test (which is true also of the cornea test) is that it should detect substances which block sensory nerve-endings, as well as those which block the axons There is no reason to suppose that the

process of hlock is the same at both these sites or that drugs are necessarily equi active at hoth, but little is known ahout this

Although activity on the spinal cord might be expected to be more closely dependent on fundamental activity than activity on the eye or in the weal test, this is not what is found The results in Tahle III 9, for instance, taken from the work of Luduena (1957) and Luduena, Hoppe, and Borland (1958), are quite different from those in other tests, including those of Skou on single nerve fibres The differences may arise from the complexity of the cord, which contains many types of fibre Fibres which synapse in the grey matter and are involved in reflex arcs are quite different in structure from fibres found in white matter Whatever may he the reason, relationships hetween structure and usefulness in spinal anaesthesia are quite different from relationships hetween structure and local anaesthetic activity and they have not heen worked out

One factor which seriously affects the usefulness of local anaesthetics in a variety of tests is their liahility to irritate or damage the tissues Luduena *et al* (1955) studied the effects of a large number of local anaesthetics on surface tension at an air/water interface, their activity, and their liahility to produce irritancy as determined by the penetration of Trypan Blue into the tissues They found that the irritation produced could he correlated with the surface activity of the drugs (whereas local anaesthetic activity could not) This finding may explain why many highly active local anaesthetics are irritant (e g the compounds studied by Fourneau and Samdahl, 1930, and hy MacIntosh and Work, 1941)

The usefulness of local anaesthetics on mucous surfaces, at nerve endings and in the spinal cord cannot really be related to chemical structure or even to physicochemical properties Usefulness can only he determined from tests made in conditions which approximate to those in which the drugs are to he used It is for these reasons that fundamental tests of local anaesthetic activity are seldom performed

Conclusion

The discussion of local anaesthetics emphasizes the importance of physico chemical effects in pharmacology and also our ignorance of the processes involved in transport mechanisms, both of ions through the nerve cell membrane and of drugs through mucous memhranes

IV

Classification of Synapses

Distinction between nerve, muscle, and synapse

It is convenient to distinguish between synapses, whether of nerve with nerve (as in ganglia) or of nerve with muscle, and nerve fibres and muscles or organs Conduction of impulses along a nerve or muscle-cell is continuous, but at the synapse conduction is interrupted

Sensory Nerve-Endings

Sensory nerve endings are quite different from motor nerve-endings They are affected by some sort of stimulus, chemical, electrical, thermal or mechanical, and give rise to an impulse in the sensory fibre Motor nerve-endings operate in the other direction and result in a mechanical response when an impulse arrives at the nerve-endings Very little is known about sensory nerve-endings and still less about the action of drugs upon them The actions of compounds upon the endings of sensory nerves responsible for taste and smell are the concern of the cook and of the flavouring and perfume industries, but the difficulties of assessing taste and smell experimentally are a great obstacle to the discussion of the relationships between chemical structure and biological activity

Some progress has been made in the study of the sensory nerve endings in the retina associated with visuo (see for example, Pitt and Morton, 1960), but the only actions of drugs on sensory nerve endings which will be considered in this book are those involving 'chemoreceptors' These lead to reflex changes in heart rate, blood pressure and/or respiration Chemo receptors located in the aorta and in the carotid body (in the neck), for example, produce a rise in blood pressure and heart rate when stimulated, in contrast, baroreceptors located in the aorta and carotid body, produce a fall in blood pressure and heart rate when stimulated These receptors appear to be concerned with the normal maintenance of an appropriate heart rate and blood pressure One set raises the blood pressure when an increase in the carbon dioxide content of the blood indicates that it is too low The other set lowers the pressure when it is too high There seem to be a number of chemoreceptors located in various parts of the cardiovascular system Veratrine

alkaloids (Dawes, 1947), for example, and amidines and isothioureas (Dawes and Mott, 1950, Dawes and Fastier, 1950, review by Dawes and Comroe, 1954) produce reflex changes in blood-pressure and heart rate by actions involving at least three sets of reflexes Two of these are depressor and the receptors are located in the heart and lungs, the third is respiratory, with receptors in the lungs

These actions will not be discussed in detail, but it is important to keep this type of effect in mind when assessing information derived from experiments on the blood-pressure or heart rate of whole animals The effects should disappear when the sensory nerves are cut

Transmission at Synapses of Motor Nerves

The arrival of an impulse at the nerve endings in a ganglion or at a synapse with a muscle-cell results in the release of a chemical substance, called a 'transmitter' It is this which acts on the next part of the chain of communications, the dendrons of the postganglionic fibre or the receptors on the muscle or organ cell These are extremely sensitive to the transmitters, much more so than the surrounding tissues

Proof of the Chemical Transmission of Impulses Across Synapses

The possibility that impulses might be transmitted across synapses by chemical mediation was first suggested by Elliot (1904, 1905) for postganglionic sympathetic nerves Progress, however, was more rapid with the investigation of parasympathetic synapses and was based on the thorough examination of the properties of acetylcholine (IV 1) by Dale (1914), which made it seem likely that this particular substance might be a transmitter Even at these synapses the problem was complicated by the rapid destruction of acetylcholine by cholinesterases, and it was not until after the discovery

$$CH_3-C\begin{smallmatrix}O\\\\O\end{smallmatrix}-O-CH_2CH_2\overset{+}{N}Me_3$$
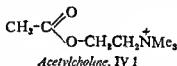
Acetylcholine, IV 1

of the effect of eserine in preserving acetylcholine from destruction that advances could really be made The first convincing evidence for the theory of chemical transmission was provided by the experiments of Loewi (1921) Two isolated frog hearts were arranged so that the perfusion fluid (a suitable salt solution) flowed from one to the other (Fig IV 1) When the vagus nerve supplying the first heart was stimulated it beat more slowly, but Loewi observed that, at the same time, the second heart was also slowed Only the perfusion fluid connected the two, therefore the effect on the second heart must have been caused by the presence of some substance (which he called the 'vagus stoff') in this fluid In suitable circumstances stimulation of the sympathetic nerve to the first heart increased the rate of beating of that heart and also of the second heart, thus showing that transmission at postganglionic sympathetic synapses involved a chemical agent (the 'accelerans stoff')

The identity of the 'vagus-stoff' with acetylcholine followed from the work of Chang and Gaddum (1933) and Gaddum (1936) Acetylcholine had been

shown by Dale and Dudley (1929) to be a substance which occurred naturally in the body (they succeeded in isolating it from ox spleen)

Chemical transmission in ganglia was demonstrated by Kibjakow (1933), who perfused the blood supply to the superior cervical ganglion of the cat (this is a fairly large object in the neck) When the preganglionic fibres were stimulated an active substance appeared in the perfusate Feldberg and Gaddum (1933) and Feldberg and Vartiainen (1934), by adding eserine to the perfusion fluid, were able to show that this was acetylcholine Feldberg and Minz (1933) showed that acetylcholine was released following stimulation of
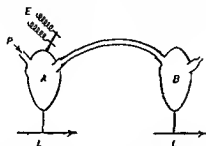
FIG IV 1   *Demonstration of the liberation of a chemical transmitter diagrammatic only*
      Perfusion fluid, P, passes from heart A to heart B Electrodes, E, used to stimulate the vagus nerve L and L are light levers which write on smoked drums

the splanchnic nerve to the adrenal medulla This structure can be likened to a sympathetic ganglion which has lost its postganglionic fibres or in which these have been reduced to zero length The release of acetylcholine, the transmitter in ganglia, produces a discharge from the adrenal medulla of Sympathin, the transmitter at postganglionic sympathetic synapses (see below)

At the neuromuscular junction, i e the synapses with voluntary muscle, chemical transmission was established by the work of Hess (1923) and acetylcholine shown to be the transmitter by Dale and Feldberg (1934) and Dale, Feldberg, and Vogt (1936)

The identity of the transmitter at postganglionic sympathetic synapses ('accelerans stoff' or sympathio) has been difficult to establish This is partly because its effects are different at different organs and partly because it is not a single substance Sympathetic stimulation of some organs produces a contraction or increased activity whereas stimulation of other organs produces relaxation or decreased activity Cannon and Rosenblueth (1933) suggested that the mediator released gave rise to a substance Sympathin E at the first type of synapse and to a substance Sympathin I (with different properties) at the second Sympathin E was supposed to cause the excitatory responses and Sympathin I the inhibitory ones This idea does not seem to be absolutely correct, nevertheless, the classification of the responses into excitatory and inhibitory is very convenient

Although, ever since the work of Barger and Dale (1910), it was known that adrenaline (IV 2) produced effects remarkably like those of sympathetic

stimulation, the parallel was not absolute Bacq (1933) had suggested that stimulation of sympathetic nerves might lead to the release of *noradrenaline* (IV 3) and this substance has been shown to be present in sympathetic nerves by Von Euler (1946), and in extracts of adrenal glands (Holtz, Credner, and Kronberg, 1947) Experiments suggest that it may be released when certain adrenergic nerves are stimulated (Gaddum and Goodwin, 1947, Peart, 1949)

The effects of *noradrenaline*, however, are not exactly the same as those of sympathetic stimulation, in particular, it is less effective than adrenaline at sites where the action is inhibitory

It is now accepted that Sympathin is a mixture of adrenaline and *nor*-adrenaline (possibly there is even a third substance involved), the propor-

HO—⟨benzene ring⟩—$\overset{\displaystyle H}{\underset{\displaystyle OH}{C}}$—CH₂NHR

HO—
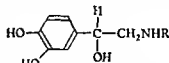
R = Me Adrenaline IV.2
R = H, Noradrenaline, IV 3

tions of which vary from one site to another The proportions may also vary with the amount of nervous activity Sympathin from the adrenal medulla contains a high proportion of adrenaline, but if the splanchnic nerve is stimulated for some time, the proportion of noradrenaline rises considerably (West, 1950) If the gland is exhausted (e g by treatment with acetylcholine) the replacement of the stores of *noradrenaline* is much more rapid than the replacement of the stores of adrenaline (Butterworth and Mann, 1957)

Chemical Classification of Synapses

Nerve-endings can be classified into cholinergic and adrenergic, according to the nature of the transmitter This chemical classification is found to correspond with some of the physiological divisions which are discussed in the Appendix, thus

Motor Nerve endings

	Physiological class	Chemical class
1 Voluntary		Cholinergic
2 Sympathetic	(a) Preganglionic	Cholinergic
	(b) Postganglionic	Adrenergic
3 Parasympathetic	(a) Preganglionic	Cholinergic
	(b) Postganglionic	Cholinergic

The splanchnic nerve to the adrenal medulla, which belongs to class 2a and has no ganglionic relay, is also cholinergic, so are some fibres to sweat-glands, although they belong anatomically to the sympathetic system Certain blood vessels dilate in response to acetylcholine, although they are not innervated by the parasympathetic These may be a further example of structures innervated by sympathetic cholinergic fibres but are more likely not to be innervated at all

Correlation of the Chemical and Anatomical Classifications of Synapses

Although acetylcholine is the transmitter at so many different types of nerve endings its effects are not the same at all of them At synapses in ganglion cells, with voluntary muscle (these synapses are called *neuromuscular junctions*), and in the adrenal medulla, its effects are immediate, highly

localized (i e nearby tissues are not affected), and extremely brief, summation (i e the fusing into one of responses to rapid stimuli) does not readily occur All these features ensure a fairly precise nervous control of the tissues At postganglionic nerve endings, whether cholinergic or adrenergic, the effects of the chemical transmitters are slower in onset, less highly localized, and more prolonged in action, summation occurs readily Nervous control of tissues innervated by postganglionic fibres (cholinergic or adrenergic) is less precise than that in ganglion cells or voluntary muscle The overall position is summarized in Table IV 1 in which the various types of nerve are classified (A, B, or C as in Table Appendix II 2)

TABLE IV 1
Classification of Nerves

Fibre	Appearance	Length	Type of response	C T	Group
Sensory	Medullated	Long	?	?	A (C in spinal roots)
Voluntary	Thick, medullated	Long	Rapid	Ach	A
SYMPATHETIC					
Preganglionic	Thin, medullated	Short	Rapid	Ach	B
Postganglionic	Thin, non medullated	Long	Sluggish	Adr	C
Splanchnic	Thick, medullated	Long	Rapid	Ach	?
PARASYMPATHETIC					
Preganglionic	Medullated	Long	Rapid	Ach	B
Postganglionic	Non medullated	Short	Sluggish	Ach	C

C T = Chemical transmitter Ach = acetylcholine Adr = Sympathin

Classification of the Actions of Drugs at Peripheral Synapses

It is necessary first to distinguish between

    1 Drugs which affect the release of the transmitter from the stores in the nerve endings

    2 Drugs which affect the action of the transmitter on the receptors on the postganglionic neurone or on the effector cell, and

    3 Drugs which affect the destruction of the transmitter and thereby prolong its effect

Until recently few drugs of the first type were known, but important examples (e g *Bretylium*, page 341) have now been discovered These act on the nerve terminals a structure quite distinct from the axon and from the muscle cell or postganglionic nerve-cell

Most drugs, like the transmitters themselves, affect the receptors on the postganglionic neurone or on the muscle-cell At the synapses of voluntary nerve with muscle these receptors are collected together in a highly organized structure called the end plate At synapses with smooth muscle, however, the receptors are distributed much more diffusely over the cell and there is no *structure corresponding to the end plate* The term 'neuromuscular junction'

is used to refer to synapses with **voluntary** muscle and this term should not be used to describe synapses with smooth muscle Although the latter are, logically speaking, junctions of nerve with muscle, the lack of organization at this junction and its different characteristics necessitate distinguishing clearly between the two types Synapses in ganglia resemble those in the neuromuscular junction more than they resemble the synapses with smooth muscle, but this type of synapse, too, must be distinguished from the others

The ability of drugs to act by preventing the destruction of the transmitter obviously depends upon the existence at the synapse of some mechanism which rapidly destroys the transmitter As will be discussed in Chapter VIII, the transmitter acetylcholine is rapidly destroyed at many synapses by cholinesterases Many drugs, such as eserine (page 259), produce pharmacological effects very like those of acetylcholine by inhibiting the actions of these cholinesterases The mechanism for the destruction of noradrenaline and adrenaline is not so clearly known, nor does it appear to be as important physiologically in limiting the action of the sympathetic transmitter as are the cholinesterases in limiting the actions of acetylcholine

When classifying the actions of drugs at synapses it is also necessary to classify the synapses themselves Just as the effects of acetylcholine are not the same at all synapses even though it may be the transmitter (see above), other drugs differ in action from one synapse to another even though these may all be cholinergic The actions of acetylcholine at synapses of voluntary connexions and in ganglia is imitated by nicotine (small doses), antagonized by curare alkaloids (and large doses of nicotine), but relatively unaffected by atropine These effects are called the nicotine-like properties of acetylcholine (Dale, 1914)

At the synapses of postganglionic parasympathetic fibres with smooth muscle the effects of acetylcholine are imitated by muscarine, abolished by atropine, and unaffected by curare alkaloids This is also true at postganglionic sympathetic cholinergic synapses These are called the muscarine-like properties of acetylcholine

Even the division of cholinergic synapses into sites of the nicotine-like actions of acetylcholine and sites of the muscarine like actions is inadequate The receptors in the ganglia and neuromuscular junction must be different even though these are both sites of the nicotine-like actions of acetylcholine The substance Decamethonium (page 109) is an effective blocking agent at the neuromuscular junction and virtually without any effect at ganglia, whereas Hexamethonium (page 165) is a ganglion blocking agent with little action at the neuromuscular junction

Reference has already been made to the differences between the ability of noradrenaline and adrenaline to produce excitatory and inhibitory responses Ahlquist (1948) suggested that the receptors producing excitatory responses be classified as 'α'-receptors to distinguish them from those producing inhibitory responses, 'β' receptors The receptors in the heart are also classified as β, but appear to be different from other β receptors and are

probably best put in a class of their own The action of *noradrenaline*, for instance, is most marked at α receptors, but the substance also acts on the β receptors in the heart

The actions of drugs at peripheral synapses can, therefore, be classified according to the type of synapses affected, the site within the synapse which is affected, and the nature of the effects, i e whether the drug produces the same effect as the transmitter or blocks transmission

The classification adopted in this book is

1 Stimulation and block of voluntary synapses
2 Stimulation and block of synapses in ganglion cells
3 Stimulation and block of postganglionic parasympathetic synapses
4 Imitation of the action of acetylcholine by inhibiting its destruction
5 Effects on postganglionic sympathetic (adrenergic) synapses

This classification, although it distinguishes some drugs which act in directly by affecting the enzyme destruction of the transmitter, is inadequate in that it does not specify the site within the synapse which may be affected by the others Most drugs act on the receptors on the muscle cell or postganglionic neurone, but some drugs act, or appear to act elsewhere within the synapse (e g on the pre synaptic nerve terminals) There are not enough clearly established examples of this type of action, however, to justify setting these out separately, and they are discussed in a section of the appropriate chapter

Production of the Same Effects by Different Mechanisms

Most organs of the body are innervated by both sympathetic and para sympathetic systems The balance can be upset in the same direction either by stimulation of the one or depression of the other It is therefore necessary, when both systems are present in the test object, to establish which is being affected by the drug This is usually done by seeing how the responses are modified by the action of an agonist or antagonist which is known to affect one system and not the other The trouble can be avoided by the selection of a test object which contains only one type of synapse, but this is not always easy Even in these circumstances there is the possibility that drugs may act at different sites within the synapse

The terms 'sympathomimetic' and 'parasympathomimetic' are often used to describe the effects of drugs which act at synapses in the autonomic nervous system This nomenclature, though conveniently descriptive, gives no indication how the effects are produced and may be misleading It is also extremely common, for example, to test the effects of drugs on blood pressure, and although this may lead to the rapid classification of a drug as sympathomimetic (pressor) or parasympathomimetic (depressor) it may be very difficult to interpret the results correctly

The blood pressure depends upon the rate of pumping of the heart the

degree of constriction in the large arteries, and the resistance of the peripheral blood-vessels The rate of pumping of the heart depends not only on the rate of beating but also on the **stroke-volume**, and the peripheral resistance depends on the degree of dilatation or constriction of not merely one type of blood-vessel but of many types Additional factors to be considered in intact animals are the reflex mechanisms which may offset any rise or fall in blood-pressure (page 78) and which can be eliminated by cutting the sensory nerves In general, the peripheral resistance is more important in determining blood-pressure than is the rate of pumping of the heart If the blood-vessels are dilated there will be a fall in pressure even though the heart is pumping hard, though in this situation there may be a big difference between the systolic pressure (at the peak of the contraction) and the diastolic pressure (when the effects of contraction are weakest) Another important point is that the sympathetic system is more important in setting the resting blood-pressure than the parasympathetic A drug which blocks conduction in ganglia (both sympathetic and parasympathetic) is likely to produce a fall in blood-pressure and likewise a drug which stimulates both types of ganglia is likely to produce a rise

A fall in blood-pressure could be produced by a slowing of the heart-rate, vasodilatation of peripheral vessels, or reflex stimulation of the vagus nerve A slowing of the heart-rate could be brought about by an acetylcholine-like action at the ganglia of the heart or at postganglionic synapses It could also be brought about by a block of sympathetic impulses either at the sympathetic ganglia or at the postganglionic synapses Dilatation of the blood-vessels could be an acetylcholine-like response or, in the blood-vessels of voluntary muscle, an adrenaline-like response at β-receptors It could also be brought about by a block of sympathetic impulses leading to vasoconstriction

A rise in blood-pressure could be produced by an increase in the rate of pumping of the heart, by vasoconstriction of peripheral vessels or reflexly The increased rate of pumping could be the result of an adrenaline like action (at β-receptors in the heart) or of a block of cholinergic parasympathetic impulses with a consequent freeing of the heart from parasympathetic inhibitory control Vasoconstriction of peripheral vessels is most likely to he the result of an adrenaline-like action at the α-receptors Blockage of cholinergic vasodilator effects is not likely to lead to much rise in pressure because, as has already been mentioned, it is the sympathetic supply which is more important in setting the blood-pressure, and there is no indication that these particular blood-vessels are innervated by adrenergic sympathetic fibres

Changes in blood-pressure may also be brought about by an action on the adrenal medulla, or reflexly by actions at chemo- or baro sensory receptors (page 78), by an action on the central nervous system, or by a direct action on the heart or the blood-vessels Ergotamine, for example, has a direct vasoconstrictor action on peripheral blood-vessels as well as its action in blocking adrenergic synapses (page 320) Changes may even be produced indirectly by an action on the coronary circulation, which supplies the heart itself, although most of the substances which affect the coronary vessels (e g

papaverine, which dilates them), also affect some, at least, of the peripheral vessels

It will be seen that the interpretation of pharmacological tests of activity on the blood-pressure may be very difficult, although the difficulties may not be apparent at first sight The cardiovascular system is an extreme example of a test object on which the same effects may be produced by a variety of different mechanisms

V

Actions at Cholinergic Synapses:
I. The Neuromuscular Junction

Acetylcholine at the Neuromuscular Junction

*Ability to stimulate the receptors in the neuromuscular junction is a nicotine-
like property of acetylcholine* The response of voluntary muscle should be a
twitch, comparable with that produced by stimulating the voluntary nerve
supplying the muscle

It is not easy to produce such a twitch, however, simply by injecting
acetylcholine Frank, Nothmann, and Hirsch-Kaufmann (1922) showed that
when the nerve supply to voluntary muscles was cut and allowed to degener-
ate, the denervated *muscles* were abnormally sensitive to acetylcholine
Gasser and Dale (1926) and Dale and Gasser (1926) found that acetylcholine,
injected intra-arterially, produced a contracture of such denervated muscles
(they used the cat's gastrocnemius), but the effects are variable (Brown, Dale,
and Feldberg, 1936) and resemble tetanic responses rather than twitches The
method of injection is extremely important, and if the acetylcholine is applied
close enough to the neuromuscular junction a response resembling a twitch

D   87

may be obtained even with normal mammalian muscle (Brown, Dale, and Feldberg, 1936)

The effects of acetylcholine are, however, most satisfactorily demonstrated by the direct application of small amounts of acetylcholine to the neuro-muscular junction Micro electrodes and micro-pipettes have been developed (Graham and Gerard, 1946, Ling and Gerard, 1949, Nastuk and Hodgkin, 1950, Fatt and Katz, 1951, Nastuk, 1951, 1953) which have a tip whose diameter (approximately 0 5 μ) is less than that of certain voluntary fibres, such as those of the frog sartorius (50–100 μ), and it is, accordingly, now possible to apply acetylcholine in conditions closely resembling the physiological release from the nerve ending. The pipette, filled with acetylcholine, is placed near the end-plate (visible under a binocular microscope of approximately 80 × magnification), and a pulse of acetylcholine is released electrophoretically. In these circumstances the muscle responds with a twitch

This mechanical response is associated with electrical changes at the membrane of the muscle end-plate. If a micro electrode is placed inside the cell at the end-plate region and a second micro electrode just outside it, there is a resting potential difference of about 90 mV, the outside being positive with respect to the inside. Stimulation of the voluntary nerve, or local electrophoretic application of acetylcholine, produced a depolarization of the membrane followed by a repolarization, the whole change being termed an end plate potential, this is followed by the action-potential of the muscle-fibre. The process can be compared with the action-potential in a nerve-fibre, and it can be supposed that the interaction of acetylcholine with the receptors in the end-plate leads to changes in the ionic permeability of the membrane and that consequent ion movements account for the end-plate potential and, possibly, also trigger off the contractile mechanism of the muscle-fibre. Whatever the mechanism whereby the union of acetylcholine with the receptors in the end-plate leads to contraction of the muscle cell, it seems clear that the electrophoretic application of acetylcholine to the end plate leads not only to a twitch comparable with that produced by acetylcholine, but also to comparable end plate potentials

Miniature End-plate Potentials

It has been found (review by Katz, 1958) that in the normal resting state the membrane potential is not absolutely steady but is disturbed by small changes, termed miniature end-plate potentials, which have the same shape as a full-sized end plate potential but are very much smaller (0 5–1 mV), and which do not lead to a twitch-response by the muscle. These changes are thought to be due to the intermittent release of small quantities of acetylcholine from stores in the nerve-endings, these stores being discrete vesicles, which are recognizable histologically (Fig V I). These ideas imply that the release of acetylcholine is quantal and this fits in with what is known about the distribution of the size of the miniature end plate potentials. These are not randomly distributed about a mean value but appear to be distributed about several values which could be taken to represent the end-plate potentials

produced by a particular (whole) number of quanta of acetylcholine It is suggested that the disruption of the vesicle through the membrane of the nerve-ending may involve Ca^{++} as a co-factor It has been estimated (Acheson, 1948) that a single (full) end-plate potential results from the release of about 5×10^5 molecules of acetylcholine at a single nerve-ending It has also been estimated that a (full) end plate potential is equivalent to between 220 and 310 (Boyd and Martin, 1956) and 250 and 500 (Liley, 1956) miniature end-plate potentials It follows that the vesicles contain about 1,000 molecules of
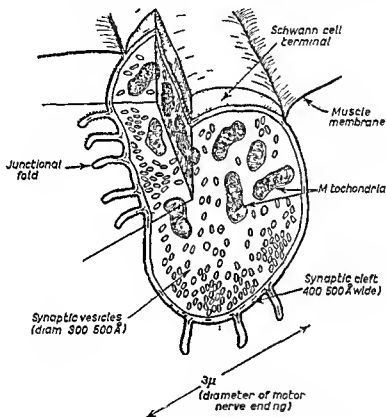
FIG V I   *Cutaway view of motor nerve terminal lying in its 'synaptic trough' in a muscle fibre Cf Birks, Huxley & Katz (1960) J Physiol , 150, 134–44*

acetylcholine MacIntosh (1959) puts the figure as low as 400 for motor nerve-endings in the cat, Straughan (1960) puts it as high as 1,500 for the endings in the rat diaphragm When acetylcholine is applied electrophoretically to produce an end plate potential, much larger amounts, about 10^8 molecules (Castillo and Katz, 1955), must be given than those released by stimulation of the nerve This is presumably because the pipette is much further from the receptors on the muscle than is the nerve-ending and only a small fraction of the electrophoretically released acetylcholine reaches the receptors Krnjevic and Miledi (1958) have obtained end plate potentials with the rat diaphragm using only 4×10^6 molecules of acetylcholine

Removal of Acetylcholine

In the resting state the receptors in the muscle end-plate are exposed to concentrations of acetylcholine which are too low to produce a response The acetylcholine molecules are lost by diffusion from the end-plate and subsequent destruction by acetylcholinesterase (page 241), which is located nearby, but not at the receptors themselves The enzymatic destruction of acetylcholine will create a concentration gradient which should assist in the removal of the compound by diffusion This process appears to be highly efficient in voluntary muscle because the response of this muscle to stimulation is remarkably rapid and transient Separate twitch responses can be obtained with repetitive stimuli at rates as high as several shocks per second and it is only with higher rates that summation occurs

Effects of Degeneration

If the nerve supply is cut and allowed to degenerate, miniature end plate potentials are no longer seen and, as already mentioned, the denervated muscle is extremely sensitive to acetylcholine It may be supposed that this extreme sensitivity arises from the absence of any acetylcholine at the receptors in the resting state of the muscle If the relationship between receptor occupancy and biological stimulus is somewhat similar to the curve on page 9, and if the tissue can accommodate itself to subthreshold stimuli maintained for a long time, the change in stimulus produced by quite small amounts of acetylcholine in denervated muscle (ΔS) would be much greater than the change produced by a comparable increase in the acetylcholine concentration where there was already a considerable concentration of acetylcholine There is also evidence, however, that the changes in sensitivity after denervation may, after several days, be due to changes in the distribution of receptors on the muscle (Miledi, 1961, cf Thesleff, 1960)

Antagonism of the Action of Acetylcholine at the Neuromuscular Junction

A substance which antagonizes the action of acetylcholine at the neuro muscular junction should lead to a block of transmission In an individual junction, consisting of a single nerve fibre and a single muscle-fibre, transmission is all or none and the effect of an antagonist will be all or none It will either interrupt transmission or fail to interrupt it With a whole muscle, containing a considerable population of end plates, an antagonist may produce a graded response, i e n partial paralysis, if the concentration of the antagonist is such that only a certain proportion of the end-plate population is blocked (although each end plate is blocked completely)

The oldest known neuromuscular blocking agents are the curare alkaloids, a group of South American arrow poisons which is divided into 'tube', 'calabash', and 'pot' curares according to the sort of vessel in which they are prepared As long ago as 1850, Pelouze and Bernard showed that the paralysis by extracts of 'curare' was caused by an action at the neuromuscular junction One leg of a decapitated frog was tightly ligatured, the curare extract was

injected into the rest of the animal, but the drug does not reach the ligatured limb (Fig V 2) When the ligatured limb was immersed in acid it twitched violently This was a reflex response sensory fibres carried impulses to the spinal cord whence motor fibres carried the efferent impulses to the muscles involved in the withdrawal process The unligatured limb, however, did not respond to immersal in acid, although it was shown in other experiments (Bernard, 1857) that 'curarized' muscles could still be made to contract by direct electrical stimulation The drug, therefore, does not act on the nerve
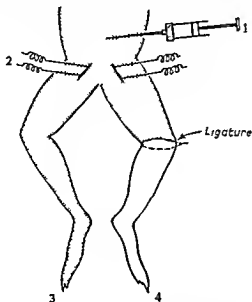
FIO V 2   1 *Drug injected into abdomen, shaded area represents the parts of the animal affected*
2. *Sciatic nerve connected to electrodes*
3 *Limb affected by drug, fails to respond to stimulation of nerve, but does respond to direct stimulation*
4 *Limb unaffected by drug, responds to electrical stimulation of sciatic nerve*

(for the nerves involved in the withdrawal of the ligatured limb are exposed to the action of the drug above the ligature), nor does it act on the muscles themselves (since these can still respond to direct stimulation), and must therefore act at the junction of the nerve with the muscle This idea was also put forward independently by Kölliker (1856)

If the electrical events at the end plate are studied it is observed that an antagonist, such as (+) tubocurarine chloride (a pure alkaloid of tube curare which is commercially available), has no action on the resting potential, but markedly reduces the end plate potential produced by stimulation of the nerve or electrophoretic application of acetylcholine (Fig V 3) It has no effect if applied inside the muscle fibre (Castillo and Katz, 1957) It is supposed that (+) tubocurarine ions compete with acetylcholine ions for receptors on the end plate membrane This assumption receives support from the work of Van Maanen (1950) and Kirshner and Stone (1951) who

tested tbe effects of different concentrations of (+)-tubocurarine in antagon-
izing the contracture of the frog rectus abdominis (see below) produced by
different concentrations of acetylcholine More direct evidence is provided
by the experiments of Jenkinson (1960) who has studied quantitatively the
effects of different concentrations of the depolarization produced by various
concentrations of acetylcholine and carbachol (page
252) The muscles tested included both slow fibres (frog rectus abdominis)
and twitch fibres (frog sartorius), and the results were consistent with the
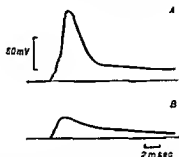
FIG V 3   *Electrical responses at the end plate of a single fibre of the frog's sartorius
muscle when the motor nerve is stimulated the responses indicate the potential
difference between a micro-electrode placed inside the muscle fibre, near the end
plate, and a reference electrode outside the cell The upper record, A, shows the
normal response, an action potential preceded by the initial end plate potential step
The lower record, B, shows the response in the presence of a concentration of (+)-
tubocurarine just sufficient to block transmission, an end plate potential alone,
whose amplitude is not large enough to initiate an action potential (Ginsborg,
unpublished)*

hypothesis that the agonist and antagonist compete on a one-to-one basis
for the receptors at the end plate This applies, however, only to the process
leading to depolarization and not to the end plate potential or the events
leading to contraction Although, in theory, it should be simple to study the
effects of the antagonist on the end plate potential produced by electro-
phoretic application of agonist it is in practice very difficult to estimate the
amount of drug released by the electrophoretic pulse, and this experiment
does not appear to have been performed

Desensitization

Competitive antagonism of acetylcholine, however, is not the only way of
blocking transmission at the neuromuscular junction It has long been
observed that smooth involuntary muscle becomes insensitive after large
doses of acetylcholine This phenomenon is termed tachyphylaxis or de
sensitization Similar effects were observed with voluntary muscle by Brown,
Dale, and Feldberg (1936) who found that, after large doses of acetylcholine
(in the presence of eserine, which prevents its destruction by cholinesterase)
the contractions of the cat gastrocnemius muscle, in response to stimulation
of the sciatic nerve, were reduced The desensitization of voluntary muscle

however, is most convincingly demonstrated by the repeated electrophoretic application of acetylcholine at a neuromuscular junction and observing the effect on the end-plate potential Even in a few minutes (Fig V 4) it may be possible to make the end-plate insensitive to acetylcholine, and substances which act like acetylcholine may have the same effects At the neuromuscular junction, therefore, it is necessary to try to distinguish between two types of
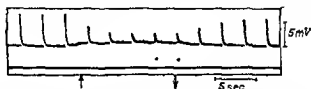
FIG V 4   *End plate desensitization produced by acetylcholine the spikes show the transient depolarization of the end plate of the tenuissimus muscle of the cat, produced by the brief electrophoretic application of acetylcholine After the three control responses acetylcholine was applied continually in a low concentration during the period indicated by the arrows and the electrophoretically applied pulses of acetylcholine produced a much smaller effect, although the resting potential of the cell (the base line) was not disturbed to any extent The responses rapidly returned to normal when the 'background' acetylcholine was washed out (Thesleff, 1958, reproduced by permission)*

action, a truly curare-like or 'pachycurare-like' competition with acetylcholine and an action like excess acetylcholine, which has been termed 'leptocurare-like' (Bovet, 1951), 'depolarizing' (Burns and Paton, 1951) or 'desensitizing' (Thesleff, 1955)

The term 'depolarizing' is rather misleading With certain drugs and certain muscles (Burns and Paton used the cat's gracilis) there is initially a depolari-
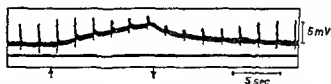
FIG V 5   *Desensitization accompanied by depolarization the experiment was similar to that shown in Fig V 4, but the end plates of the cat's gracilis muscle were used The addition of Decamethonium to this preparation produced depolarization of the end plate (shown by the rising base line) as well as a decline in the response to brief electrophoretically applied pulses of acetylcholine Note, however, that the depolarization passed off more rapidly than the desensitization when the Decamethonium was washed out (Thesleff, 1958 reproduced by permission)*

zation of the end plate This in itself could account for the neuromuscular block This depolarization, however, may pass off before the block (Fig V 5) In some muscles, the cat's tenuissimus, for instance (Thesleff, 1958), the depolarization is so small that it should not give rise to block, a comparable depolarization produced by increasing the concentration of K^+ ions does not affect transmission The block is better called a desensitization, although even

this term, which is used here, does *not* completely describe the situation because the initial stages of block may in certain circumstances be due to depolarization.

Uses of Neuromuscular Blocking Agents

Substances which block transmission at the neuromuscular junction and cause paralysis are of practical value in certain conditions. It has been observed that in certain mental diseases, such as schizophrenia, there is an improvement after the patient has been made to experience violent convulsions. These are usually induced by application of suitable electric shocks to the head. The convulsions, however, may easily result in broken or dislocated limbs, and this can be avoided by the administration of a neuromuscular blocking agent (Bennett, McIntyre, and Bennett, 1940). Likewise, in anaesthesia, before the surgeon can operate, the anaesthetist must produce relaxation. It is possible to do this with only one agent, such as diethyl ether, provided enough is given to block synapses in the spinal cord between the sensory and motor branches of reflexes. This entails using a lot of anaesthetic, however, and if a separate drug is used for producing relaxation, only enough general depressant need be given to produce unconsciousness. The central nervous system is accordingly much less disturbed. This is generally regarded as being better for the patient, especially if he or she is elderly. The use of a neuromuscular blocking agent for producing relaxation was first described by Griffith and Johnson (1942) and is now a standard procedure in anaesthesia.

Testing of Agonists at the Neuromuscular Junction

Substances which act like acetylcholine at the neuromuscular junction may produce either stimulation or block, depending on the concentration, on their ability to stimulate, and on their ability to desensitize. The most direct method of finding out how a particular drug is acting is to apply it electrophoretically at a single neuromuscular junction at which electrical events are recorded by electrodes inside and outside the cell. In practice, substances are seldom tested in so fundamental a manner, and it is, anyway, difficult to obtain quantitative comparisons in such experiments, because of the difficulty of estimating how much of the drug is released by the electrophoretic pulse.

To obtain estimates of acetylcholine-like activity at the neuromuscular junction, drugs have been tested for their ability to cause a contracture of denervated voluntary muscle (Gasser and Dale, 1926, Simonart, 1932), but the value of the results must clearly depend greatly on the closeness of the site of injection to the end plate (page 87). Experiments have also been made with normal muscle using close intra-arterial injections after the manner of Brown, Dale, and Feldberg (1936). In these the ability to cause a twitch may also be tested while the muscle is being stimulated through the voluntary nerve at a steady rate (usually 6 shocks per minute). The rapid close intra-arterial *injection* of the drug takes the place of one of the stimuli. This has the advantage that it reveals not only an acetylcholine-like stimulant

action (a twitch), but also shows the possible effect of the injection of the drug on subsequent stimuli and should accordingly reveal blocking activity

It is blocking activity at the neuromuscular junction which may be of practical value, and many compounds have been tested only for this property (see below) on such preparations as the frog sciatic nerve gastrocnemius, or femoral nerve-sartorius, the rat phrenic nerve diaphragm, and the tibialis, soleus, quadriceps, or gracilis muscles of cats or rabbits stimulated through the appropriate nerve In all these tests the ability of a drug to cause a twitch may be overlooked Even if the route of injection is such that a twitch is produced, this will be superimposed on a record consisting of maximal twitches produced by stimulation of the nerve, and all that may be observed is a slight supra maximal contraction after the injection If the dose is such that a block is produced, this effect could easily be mistaken for an antagonism of acetylcholine (page 90) rather than an action like it

It is possible to obtain some information about acetylcholine-like activity at the neuromuscular junction quite simply by testing compounds on slow-contracting muscle fibres, such as those of the frog rectus abdominis (Langley, 1907, 1913), leech muscle (Minz, 1932), or certain avian muscles These slow-contracting muscles are different from normal voluntary muscles in that they do not possess an organized end-plate and produce a slow contracture rather than a quick twitch when stimulated They are, however, striated, as distinct from smooth, fibres They also differ from normal voluntary muscles in that it is difficult to desensitize them and consequently acetylcholine-like activity is indicated by contracture, not obscured by any desensitization block This contracture is associated with depolarization of the cell membrane (Ginsborg, 1960) If a compound produces contracture of such slow fibres it seems to be likely that it will act like acetylcholine at the normal neuromuscular junction, giving rise to end-plate potentials and, probably, to desensitization

Buttle and Zaimis (1949) used whole chickens in a qualitative test to distinguish between acetylcholine like neuromuscular blocking agents and truly 'curare like' substances The former caused a spastic paralysis in which contracture of the muscles of the back of the neck pulled the head upwards and backwards, whereas the latter caused a flaccid paralysis in which the head fell forwards and downwards Ginzel, Klupp, and Werner (1951) used the gastrocnemius of the pigeon for testing ability to cause contracture and Thesleff and Unna (1954) observed that, on the chicken gastrocnemius preparation, some neuromuscular blocking agents produced a contracture at the same time as reducing the size of the twitch response to stimulation of the nerve The semispinalis muscle in the back of the neck of the chick has been used for measuring ability to cause contracture (Child and Zaimis, 1960), but of all preparations the isolated chick biventer cervicis (Ginsborg and Warriner, 1960) is probably the simplest and most convenient This is a mixed muscle containing both twitch and slow fibres and so can be used (like the chicken gastrocnemius) to observe the effects of a drug on both types of fibre at once Contracture is indicated by movement of the base-line

and block by a decrease in the size of the contractions produced by electrical stimulation of the voluntary nerve supply (Fig V 6)
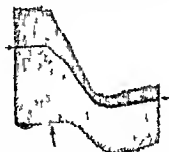
FIG V 6   *Chick biventer cervicis preparation the twitch responses to electrical stimulation are the downward contractions from the base line (the position of the base line is indicated by the horizontal arrows the portion of the trace above it is caused by the action of the return spring on the lever) Decamethonium was added at the arrow and produced a marked contracture, shown by the shift of the base-line, with little effect on the twitch response (Zoller, unpublished)*

Testing of Antagonists at the Neuromuscular Junction (i e. Neuromuscular Blocking Agents)

Antagonistic activity is most fundamentally expressed by the antagonist constant K_B (page 43) The measurement of pA_2, for example, is a simple way of determining this This is easy if the agonist can be applied in a controlled fashion, as is the situation when acetylcholine is added to an isolated muscle, such as the frog rectus, giving rise to a contracture To obtain twitch responses, however, the acetylcholine cannot be added in this way and must be applied at least close intra arterially or electrophoretically at the end plate, and even then it is difficult to be sure what the concentration at the end plate really is In theory, nevertheless, K_B could be determined by measuring the concentration of antagonist which necessitated doubling the concentration of acetylcholine in order to obtain the same biological response as before the antagonist was added Difficulty also arises over what is taken as the biological response A twitch is an all or-none response, and in this kind of experiment it is much easier to work with a graded response (page 33) The end plate potential would be a more convenient measure, but this involves recording with an intracellular electrode Jenkinson (1960) has used the effect of the antagonist on the depolarization of the muscle membrane, recorded externally, and obtained values of K_B for (+) tubocurarine of 3 1 × 10⁶ on the frog rectus and 2 3 × 10⁶ on the frog extensor longus digitorum IV muscle In general, however, the activity of antagonists at the neuromuscular junction has been assessed by methods which yield much less fundamental information, but which may give some indication of the possible value of the compounds as relaxants

Preparations used for testing the actions of neuromuscular blocking agents can be divided into those in which the muscle is caused to contract by

electrical stimulation of the appropriate voluntary nerve, and those in which the stimulus is provided by the animal itself in response to its surroundings It is further necessary to distinguish between isolated and intact preparations

In all isolated preparations, e g the frog sartorius, the phrenic nerve-diaphragm preparation of the rat (Bulbring, 1946), and other species (mouse, kitten, and guinea pig), and the chick biventer cervicis, the muscle is made to contract by electrical stimulation of the nerve Square-wave pulses of short duration are used to ensure single twitch responses from the muscle if the duration of the stimulus is longer than about 1msec multiple responses or tetanic contractions may be obtained The preparation is mounted in a bath to which the drug can be added (Fig II 9, page 40) This technique can only be employed when the muscle is thin and the drug can diffuse easily to the end plate The frog gastrocnemius, for instance, is too thick to be mounted satisfactorily in this way Although the preparation can be kept alive for some time and appears to be physiologically sound, the rate of diffusion of drugs from the bath into the muscle (which will vary inversely as the square root of the molecular or ionic weight) affects estimates of neuromuscular blocking activity (Ing and Wright, 1931) Only when this preparation is perfused and the drug added to the perfusion fluid are results obtained which are comparable with those found with the (thin) frog sartorius muscle, either perfused or in a bath

In most mammalian nerve-muscle preparations (except the rat diaphragm) the circulation of the animal is left intact The animal is anaesthetized (often the brain is destroyed) and the appropriate limb fixed rigidly in a myograph stand The most common muscles which have been used are the tibialis, soleus, gastrocnemius, quadriceps, and gracilis muscles of cats or rabbits stimulated through the appropriate nerve Before the drug is given, the electrical stimuli should give rise to contractions of almost identical height The effect of the neuromuscular blocking agent should be to cause a decrease in the height followed by a return to the original size as the effect of the drug wears off (Fig V 7) The site of injection of the drug may be very important If it is injected into the femoral vein of the opposite leg it must pass through practically the whole circulation before the drug reaches the muscles from which records are being taken The amount of drug reaching the site of action will accordingly be much less than the amount injected, partly because the dose will have been greatly diluted and partly because it may have been appreciably destroyed by the liver or excreted by the kidneys If, on the other hand, the injection is made intra arterially, close to the muscle being stimulated, there will be little opportunity for dilution or destruction and quite different results may be obtained

To approach as nearly as possible to the clinical situation it is necessary to do away with the artificial electrical stimulation of the muscle In the method of Marshall (1916), drugs were injected into frogs and these were considered to be paralysed when they could no longer right themselves when placed on their backs In the head drop test (Varney, Linegar, and Holaday, 1948), the drug is infused at a standard rate into the ear vein of a rabbit until it can no

longer hold its head up (this is quite a sharp end point) The infusion is then stopped, and the amount of drug administered is recorded Smith, Pelikan, Maramba, and Unna (1953) have described a test which is similar in principle, it depends on the ability of mice to stay on a screen inclined at 60° to the horizontal Groups of mice are given a dose of the drug and the percentage paralysed is recorded The graph of the probit of this percentage against log

Drug A *x moles*   Drug B *y moles*   *Time in m nutes*
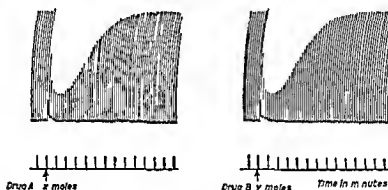
FIG V 7   *Comparison of neuromuscular blocking agents*
   The contractions of the muscle, in response to stimulation of the nerve are recorded upwards The injection of a drug causes a decrease in the size of this twitch, but this effect passes off as the drug is lost by dilution and excretion In an ideal experiment the dose, x Moles, of one drug produces exactly the same response as the dose, y Moles, of the second Note the evidence in this particular tracing that the compounds produce depolarization (the increased height of the contraction immediately after the injection and the disturbance of the base line, indicating some contracture)

dose is taken to be linear (page 38) Experiments of this type can be extended to human volunteers (Organe, Paton and Zaimis, 1949) a dose is given which should produce relatively small effects, and the degree of paralysis of the muscles of the hand is used to assess the effects of the drug This can be expressed quantitatively as a percentage of the normal grip-strength, recorded with a dynamometer or by compressing a bulb attached to a manometer (Mushin *et al*, 1949, Unna *et al*, 1950, Bodman, 1952)

Information Obtainable from Tests

The determination of the relative activities of different drugs using any of these test preparations should be made by comparing the amounts of the drugs which produce comparable effects This term 'effects' includes all the events following the administration of the drug until the preparation returns to normal In the bio assay of an unknown solution of tubocurarine against a standard sample, it is sufficient to compare the degrees of paralysis produced after any arbitrary time interval, but when two different drugs are being studied it must be established that they can, in fact, produce identical action time curves (Fig V 7) The measurement of relative activity also depends upon the log dose–response curves for the compounds being parallel

Most of the preparations described above are capable of showing graded responses (the paralysis is not all-or-none) and can, therefore, be used reliably to assess relative activity or to establish that a comparison of activity is intrinsically impossible Laidlaw (1913), for instance, showed that the activity of the optical isomers of canadine and tetrahydroberberine methochlorides could be satisfactorily expressed in terms of potency ratios by comparing the action–time curves produced by different doses of these compounds on the frog's sciatic gastrocnemius preparation Isolated preparations, however, have not always been used in this way, comparisons being made only after a particular time interval, and in the experiments with the righting reflex of frogs or in the head-drop test in rabbits the time factor is left out altogether

The 'head-drop dose' in rabbits has been used extensively to compare the relative activities of drugs Because the injection is made intravenously it is reasonable to assume that differences in the rate of onset of paralysis are not likely to be large enough to affect the results, unless the infusion is extremely slow and the drug exceedingly rapidly metabolized The 'head-drop dose', however, yields no information about the duration of action of the compound, and consequently comparisons of activity based on the 'head-drop dose' may even be misleading, implying that a comparison has been made where it is actually impossible to make one In experiments using the human grip-strength, the effects of the drugs are observed over a period of time, and this test, therefore, is much more suitable for comparing the relative activities of compounds, which can be done by comparing the amounts required to produce identical effects on the grip strength

Whether the log dose–response curves are parallel or not may also depend upon whether the drugs being tested are all acting in the same way, although, even if they are, it does not follow that the curves must be parallel, because differences in distribution and/or metabolism may result in their effects having a different time-course The sensitivity of different muscles to different drugs also varies considerably and again this may depend upon their type of action In general, the variation in sensitivity to competitive blocking agents, such as (+)-tubocurarine, is very much less than is the variation in sensitivity to acetylcholine-like desensitizing blocking agents Paton and Zaimis (1949), for instance, showed that sensitivity to (+)-tubocurarine decreases in the order

$$\text{rat} > \text{mouse} > \text{rabbit} > \text{cat}, \frac{\text{activity in cat}}{\text{activity in rat}} = 0\ 5,$$

whereas sensitivity to Decamethonium, an acetylcholine-like desensitizing drug (page 109), decreased in the order.

$$\text{cat} > \text{man} > \text{rabbit} > \text{monkey} > \text{mouse} > \text{rat}, \frac{\text{activity in cat}}{\text{activity in rat}} = 200$$

Estimates of the potency of Decamethonium relative to that of (+)-tubocurarine may vary by as much as a factor of 100, from about one-tenth as

active on rat muscle to about ten times as active on cat muscle Different muscles from the same animal may also differ in sensitivity white muscles are less sensitive than red muscles to acetylcholine-like desensitizing drugs and become more insensitive when doses are repeated Comparisons of neuromuscular blocking activity based on results from a single type of test are, accordingly, not very informative It is important for practical reasons, as well as from mere scientific curiosity, to try to discover the type of action of any compound being tested

Even if nothing is known about the action of the compound on the resting potential or end plate potential, it may still be possible to obtain some information about the mode of action by less direct means If the compound causes contracture of slow fibres there is good ground for believing that block at the neuromuscular junction of twitch fibres will be brought about by an acetylcholine like desensitization There are many compounds, however, for which estimates of neuromuscular blocking activity have been obtained, but even this test has not been done In some instances it has been observed that there are twitches of the muscles immediately after the injection of the drug and before the onset of block, this is fairly good evidence that the block is an acetylcholine like desensitization It does not follow that the failure to observe twitching can be taken as proof that the action is competitive Sometimes the reversal of block by an inhibitor of acetylcholinesterases (Chapter VIII) has been taken to indicate a competitive curarelike blocking action The anticholinesterase will allow the concentration of acetylcholine at the end plate to increase and accordingly more acetylcholine should complex with the receptors This reversal, particularly during the early stages of the action of the blocking drug is good evidence that the action is curare-like, but it does not follow that the failure of an anticholinesterase to reverse the blocking action of a drug means that its action is necessarily by desensitization Some neuromuscular blocking agents have appreciable anticholinesterase activity themselves In a similar way, the temporary increase in the acetylcholine concentration caused by a burst of rapid stimulation of the nerve should have the same effects on neuromuscular block as does an anticholinesterase The block produced by a competitive blocking agent is also relieved by an increase in the concentration of K^+ ions at the end plate, whereas the block produced by an acetylcholine like desensitizing drug is not

AGONISTS

Introduction

In the following account of substances which act at the neuromuscular junction, consideration is first given to substances which act like acetylcholine This is taken from two types of experiment, the first being tests on slow fibres (usually those of the frog rectus) and the second being tests in which neuromuscular blocking activity has been measured and there is evidence (of varying degrees of reliability) that this indicates an acetylcholine like desensitization In the latter part of the Chapter consideration is given to

substances which antagonize the actions of acetylcholine at the neuro-muscular junction

Compounds Closely Related to Acetylcholine

Simple Onium Salts

The effects of alkyltrimethylammonium salts were studied qualitatively by Boehm (1908) and Külz (1923) Ing and Wright (1931, 1933) compared the effects of equimolar solutions of these compounds on the frog rectus, but the first quantitative comparison, on this preparation and on the leech muscle, was made by Raventos (1937) Although these results (Table V 1) were based on the value tetramethylammonium = 100, and not referred to acetylcholine = 1, the contemporary experiments of Clark and Raventos (1937) show that approximately 100 times as much tetramethylammonium was required to produce the same effect as acetylcholine on this preparation, consequently the figures in Table V 1 can be taken as equipotent molar

TABLE V 1

Ability to Cause Contracture of Slow Fibres (Equipotent molar ratios)

	Leech muscle R	Frog rectus		
		R	A, S and de G	A and de G
Me₃-ṄMe	100	100	400	200
—Et	200	400	400	
—n-Pr		600	200	
—n Bu	50	25	25	32
—n Pent	80	t00	35	
—n Hex	100	200*	t00*	
—n Hept	80	t00*	250*	

* Partial agonist

In the experiments of Raventos Me₄Ṅ was taken as standard (= 100) it appears that the equipotent molar ratio for this compound relative to acetylcholine on the frog rectus in these experiments was about 100 (Clark and Raventos 1937), so the figures in the table may be taken to indicate the equipotent molar ratio

R = Raventos (1937)   A and de G = Ariens and de Groot (1954)   A, S, and de G = Ariens, Simonis, and de Groot (1954)

ratios relative to acetylcholine Included in the Table are the more recent results of Ariens, Simonis, and de Groot (1955) The value for n pentyltrimethylammonium varies considerably Ing, Kordik, and Tudor Williams (1952) obtained a value of 13 on the frog rectus In the experiments of Ing and Wright the responses of n-butyltrimethylammonium had a quicker rate of onset than those of the other alkyltrimethylammonium salts, and this difference in the rate of action might account for some of the discrepancies

Willey (1955) obtained a value of 73 for n pentyltrimethylammonium tested by close intra-arterial injection into the soleus or gastrocnemius muscles of the cat.

It appears, therefore, that activity is maximal in the n-butyl and n-pentyl members of this trimethylammonium series, and above this there is a transition through partial agonists to antagonists

Philippot and Schlag (1956) have tested the effects of these compounds on the demarcation (injury) potential of the cat's gracilis muscle The results, which should indicate ability to depolarize the neuromuscular junction, show that activity is maximal in n pentyltrimethylammonium n butyltrimethylammonium was less than half as active and n-hexyltrimethylammonium less than one-sixth as active

The replacement of the methyl groups in tetramethylammonium by ethyl groups leads to a decrease in ability to cause contracture Complete comparative figures are not available, but tetraethylammonium is inactive on the frog rectus (Ing and Wright, 1933), except possibly in concentrations greater than 1 mM (Van Rossum, 1958) Alkyltriethylammonium salts were also found to be only feebly active on the cat's gracilis (Philippot and Schlag), but nevertheless n pentyltriethylammonium (10 doses about 100 times those of the same dose of n-butyltriethylammonium or n hexyltriethylammonium) produced slightly more depolarization than the same dose of n-butyltrimethylammonium or n hexyltrimethylammonium

Ing and Wright (1933) showed that ability to cause contracture is also reduced by replacement of part of the molecule by pyridine (as in pyridine metho- and etho salts), piperidine (as in dimethyl and diethyl piperidinium salts), quinoline (as in quinoline metho and etho salts), and tetrahydroquinoline (as in dimethyl and methylethyl tetrahydroquinolinium salts) It is also reduced by increasing the size of the onium atom, tetramethylphosphonium is less active than tetramethylammonium but more active than tetramethylarsonium Trimethylsulphonium is almost as active as tetramethylammonium The ethyl analogues of all these compounds, however, do not cause a contracture of the frog rectus in concentrations up to 10^{-2} M

These substances which cause a contracture of slow fibres may also cause a block at the neuromuscular junction of twitch fibres Tetramethylammonium, tetramethylphosphonium, and trimethylsulphonium, for instance, all paralyse the frog sartorius preparation, the time taken to produce complete block with 10^{-3} M solutions being 5 8, 17, and 16 minutes respectively (Ing and Wright, 1933) Tetraethylammonium is inactive, but blocking activity re appears in tetra n-propylammonium and tetra n butylammonium, the latter being almost as active as tetramethylammonium Likewise tetra n-butyl phosphonium is almost as active as tetramethylammonium and so is tetra n-propylarsonium (even though tetramethylarsonium is only feebly active either as a blocker or in producing contracture) As these onium salts with large alkyl groups do not cause contracture, it seems that their blocking action is likely to be a true antagonism of acetylcholine and not an action like that of excess acetylcholine

Effects of Alteration of the Onium group in Acetylcholine

The effects on activity of altering the substituents on the onium group in acetylcholine, or the size of the central atom, are shown in Table V 2

These greatly resemble the effects on activity of such changes in simple onium salts, discussed above The ability to cause contracture of the frog rectus seems to be limited to those molecules in which the overall size of the onium group is small It is a pity that the tertiary and secondary bases analogous to acetylcholine do not seem to have been tested on a preparation of this type

TABLE V 2
Contracture of the Frog Rectus

	Equipotent molar ratio	
$CH_3COOCH_2CH_2\overset{+}{-}NMe_3$	1	
$-\overset{+}{N}Me_2Et$	5	
$-\overset{+}{N}MeEt_2$	300	
$-\overset{+}{N}Et_3$	5 000	
$-\overset{+}{P}Me_3$	6	
$-\overset{+}{S}Me_2$	15	
$-\overset{+}{A}sMe_3$	37	
Size of quaternary atom		

$$\underset{d}{\overset{\overset{\displaystyle X}{|}}{C}}\overset{d}{\longleftrightarrow}C$$

N	$d = 1\ 47$	$d = 2\ 4$
P	$1\ 87$	$3\ 05$
S	$1\ 82$	?
As	$1\ 98$	$3\ 23$ Å

Welch and Roepke (1935) Holton and Ing (1949) Ing Kordik and Tudor Williams (1952)

Effects of Altering the Acyl Group

The relative acetylcholine-like activities of a number of esters of choline are shown in Table V 3 Many aliphatic esters of choline are more active than acetylcholine itself, but aromatic esters are generally much less active

Some of the more active compounds can block transmission across the neuromuscular junction (Table V 4) This action might be directly related to their ability to act like acetylcholine, but this does not seem to be absolutely true It has been shown that murexine (V 1), for instance, depolarizes the neuromuscular junction of the cat's gracilis muscle (Keyl and Whittaker, 1958) and the frog's extensor longus digitorum IV muscle (Quilliam, 1957),
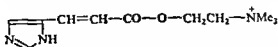
$$\underset{N}{\overset{NH}{\rightthreetimes}}-CH{=}CH{-}CO{-}O{-}CH_2CH_2{-}\overset{+}{N}Me_3$$

Murexine V 1

TABLE V 3

Activity of Esters of Choline on the Frog Rectus Equipotent Molar Ratios

ROCH₂CH₂N⁺Me₃	C and G	Wur	Wil	K, M, and W	H and W	E and G	T	O
R =								
H (choline)	700	—	—	—	—	—	—	—
CH₃CO (acetyl)	1	1	1	1	1	1	1	1
CH₃CH₂CO	0.2	0.7	0.61	0.62	—	—	—	—
CH₃(CH₂)₂CO	10	0.1	(1.1)	1.0	—	—	—	—
CH₃(CH₂)₃CO	4	—	1.0	1.9	—	—	—	—
Me₂CHCH₂CO	—	—	0.95	2.9	18	—	—	—
CH₃CH₂CHMeCO	—	—	—	0.72	—	5	—	—
Me₃CCO	—	—	—	—	—	1,000	—	—
CH₃COCO (pyruvyl)	8 (?)	—	69	—	—	25	4	—
H₂NCO (carbamoyl)	5.5	—	(4.5)	—	—	14	0.76	—
MeCH=CHCO (crotonyl)	—	—	—	4.5	63	10	7	—
Me₂C=CHCO (dimethylacryloyl)	—	—	—	5.0	1.4	29	—	—
EtCH=CHCO (pent-2-enoyl)	—	—	—	—	2.2	—	—	—
⬠⟨CO —CH₂CO	—	—	—	—	—	—	—	—
—CH₂CO	—	—	—	0.33	—	—	—	—
—CH₂CH₂CO	—	—	—	10	—	—	—	—
—CH=CHCO (muconoyl)	—	—	—	—	—	—	—	—
—(CH₂)₂CO	—	—	—	—	12	12	—	—
⟨benzene⟩—CH=CHCO	—	—	—	—	—	—	—	—
—CH₂CH₂CO	—	—	—	—	—	0.7	—	—

CH=CHCO / CH₂CH₂CO	—	—	—	—	—	25 77	—	—
CH=CHCO / CH₂CH₂CO	—	—	—	—	—	87 13	—	—
CH=CHCO / CH₂CH₂CO	—	—	—	17	—	50 11	—	—
CH₂CH₂CO	—	—	—	71 100	—	—	—	—
PhCO	—	—	21	100	—	—	—	—
m NO₂ benzoyl	—	—	—	—	—	—	—	300
m Cl benzoyl	—	—	—	—	—	—	—	400
p-Cl benzoyl	—	—	—	—	—	—	—	430
p F benzoyl	—	—	—	—	—	—	—	200
m Me benzoyl	—	—	—	—	—	—	—	4.8
p MeO benzoyl	—	—	—	—	—	—	—	430

The figures in parentheses are for experiments in the presence of eserine. Pure pyruvylcholine has been prepared by Gill (1962) and is not very active, the sample used by Chang and Gaddum appears to have been mostly impure acetylcholine. The figure 10 for murexine was obtained by Erspamer and Glasser (1957) and has been assumed in calculating the ratios for the other compounds in this column because Erspamer and Glasser (1958) expressed their activity relative to murexine in addition to the other estimates shown for murexine. Quilliam (1957) obtained the value 8

The figures for the substituted derivatives of benzoylcholine have been calculated using the figure of 100 for benzoylcholine itself. Ackasu, Sinha, and West, 1952, obtained ratios of from 5 to 20 for benzoylcholine on chick muscle

C and G = Chang and Gaddum (1933)   Wur = Wurzel (1959, 1960)   Wil = Willey (1955)   K, M, and W = Koyl, Michaelson, and Whittaker (1957),
H and W = Holmstedt and Whittaker (1958), E and G = Erspamer and Glasser (1958)   T = Tabachnick et al (1958), O = Ormerod (1956)

but β-(α-pyridyl) propionylcholine (V 2) is much less active as a blocking agent than its high activity in producing contracture would lead one to expect Part of this difference might be ascribable to different susceptibilities to hydrolysis by cholinesterase The compounds are all esters, and the

V.2
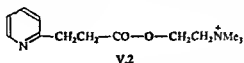
duration of their neuromuscular blocking effects certainly depends upon their stability, particularly when exposed to the cholinesterases present in plasma Holmstedt and Whittaker (1958), for instance, found that the activity of their compounds was potentiated by eserine (an anticholinesterase, page 259) It may also be noteworthy that the neuromuscular blocking activity of β-(α-pyridyl) propionylcholine is lowest in the rabbit head drop test, in which the drug is exposed to the plasma for a long time before it reaches the muscles of the neck

TABLE V 4

Neuromuscular Blocking Activity of Esters of Choline Equipotent Molar Ratios Relative to Murexine

ROCH₂CH₂N⁺Me₃	Cat gastrocnemius		Rabbit head-drop
	H and W	E and G	E and O
R =			
Me₂C=CHCO	1 5	2 0	1 7
MeCH=CHCO	1 6	—	—
EtCH=CHCO	1 9	—	—
[imidazolyl]—CH₂CO	—	1 6	4 0
—CH=CHCO (murexine)	1 0	1 0	1 0
—CH₂CH₂CO	—	0 30	0·22
—CH₂CH₂CH₂CO	—	0 77	0 61
[pyridyl]—CH CHCO	—	1 0	0 91
—CH₂CH₂CO	—	1 1	>3 3
Succinylcholine	—	0 20	0 27

H and W = Holmstedt and Whittaker (1956) E and G = Erspamer and Glässer (1958)

It is still necessary to consider, however, whether the neuromuscular block produced by desensitization is directly related to ability to depolarize the end plate like acetylcholine and cause contracture Willey (1955) found that some of the substances which caused a twitch of the cat gastrocnemius and soleus muscle, when injected close intra arterially, also reduced the size of subsequent responses of the muscle to electrical stimulation of the nerve

This block only lasted for a very short time, but whereas the most active stimulant was propionylcholine (Table V 3), the most active blocker was *n*-valerylcholine Perhaps a measure of stability as well as ability to act like acetylcholine is necessary for the production of desensitization

Effects of Altering the Choline Part of Acetylcholine

(±)-Acetyl-α-methylcholine (V 3) was found by Simonart (1932) to be slightly less active than acetylcholine in producing a twitch of the cat's denervated

$$CH_3-CO-O-CHR-CHR-\overset{+}{N}Me_3$$
R = H, R = Me, V 3,
R = Me, R = H, V 4
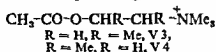
gastrocnemius muscle (equipotent molar ratio, 2) (±)-Acetyl-β-methylcholine (V 4), on the other hand, was much weaker (equipotent molar ratio, 100) Wurzel (1959) obtained the ratio 180 on the frog rectus

Acetylthiocholine (V 5), also, is less active than acetylcholine, Wurzel

$$CH_3-CO-S-CH_2CH_2\overset{+}{N}Me_3$$
V 5
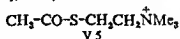
(1959) obtained a ratio of 20-40 on the frog rectus Acetyl-β-methylthiocholine has been tested on a number of sites of the nicotine-like actions of acetylcholine (page 151), but it does not appear to have been tested for its ability to act like acetylcholine at the neuromuscular junction Similarly, although the effects of altering the chain length in the choline part of acetylcholine have been studied at ganglia and at postganglionic parasympathetic synapses (Chapters VI and VII), there is little information about how such changes affect activity at the neuromuscular junction The effects of altering the positions of the carbonyl and ether groups, however, have been studied systematically by Ing, Kordik, and Tudor Williams (1952) and by Willey (1955) and are shown in Table V 5 It seems that the carbonyl part of the ester group is much more important than the ether link, the ethers are, in general, rather feebly active The activities of substituted phenyl ethers of choline, however, vary greatly with the substituent, whereas substituted phenyl esters of choline do not differ greatly in activity

Nicotine and Related Compounds

The action of nicotine (V 6) at the neuromuscular junction has already been mentioned in the classification of cholinergic synapses (Chapter III) Small amounts of nicotine cause a contracture of slow-fibres, such as the frog rectus or chick biventer cervicis, and a twitch of voluntary muscle, such as the cat gastrocnemius when injected close intra-arterially Values of the equipotent molar ratio relative to acetylcholine are 4 5–11 (Erspamer and Glasser 1960) for the frog rectus, and 24 for the cat gastrocnemius (Willey, 1955) Larger doses cause a block at the neuromuscular junction and extremely high doses will block the contracture produced by acetylcholine Of the analogues of nicotine, pyridylmethyl and ethyldialkylamines and their metho salts

Nicotinium ion,
V 6
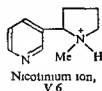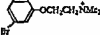
TABLE V 5

Activity of Compounds Related to Acetylcholine Ability to Cause Contracture

	Equipotent molar ratios relative to acetylcholine on	
	Frog rectus	Cat gastrocnemius (twitch)
$CH_3CH_2CH_2OCH_2CH_2\overset{+}{N}Me_3$	27	155
$CH_3CH_2OCH\cdot CH_2\overset{+}{N}Me_3$	42	—
$CH_3OCH_2CH_2\overset{+}{N}Me_3$	23	—
$n\ BuOCH_2CH_2\overset{+}{N}Me_3$	—	415
(phenyl)$OCH_2CH_2\overset{+}{N}Me_3$	—	150
Cl-(phenyl)$OCH_2CH_2\overset{+}{N}Me_3$	—	1,000
(phenyl, br)$OCH_2CH_2\overset{+}{N}Me_3$	—	5 6
$CH_3CH_2CH_2COCH_2\overset{+}{N}Me_3$	153	—
$CH_3CH\cdot COCH_2CH_2\overset{+}{N}Me_3$	1 3	—
$CH_3COCH_2CH_2\overset{+}{N}Me_3$	1 1	7 1
$CH_3CH_2COCHMeCH_2\overset{+}{N}Me_3$	700	—
$CH_3COCH\cdot CH_2\overset{+}{N}Me_3$	0 7	—
$CH_3CH_2CH\cdot CH\cdot CH_2\overset{+}{N}Me_3$	1 3	7 3

Ing, Kordik, and Tudor Williams (1952) Willey (1955)

(V 7), studied by Barlow and Hamilton (1962), only β-pyridylethyltrimethyl-ammonium (V 8) was more active than nicotine on the chick biventer (equipotent ionic ratio, 0 39) This compound bears an interesting resemblance

$(CH_2)_n - NR_2$ and $-\overset{+}{N}R_2Me$
$n = 1$ or 2
$NR_2 = NMe_2$

V 7

V 8 — $CH_2CH_2\overset{+}{N}Me_3$

Leptodactyline, V.9 — $CH_2CH_2\overset{+}{N}Me_3$ (OH)
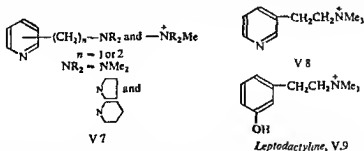
to Leptodactyline (V 9), a substance found by Erspamer (1959) to occur in the skin of a lizard, and which causes contracture of the frog rectus and also block at the neuromuscular junction The equipotent molar ratio relative to acetylcholine is 0 11–0 14, but in the same experiments values of 0 9–1 5 were obtained for murexine and 4 5–11 for nicotine These are rather different

from those obtained in other experiments (Table V 3) in which murexine appeared to be less active (equipotent molar ratio, 7–10) The blocking activity of leptodactyline, estimated by comparing doses producing roughly comparable effects on the cat gastrocnemius, appears to be about twice that of murexine

Various substituted derivatives of leptodactyline have been prepared and studied by Glässer and Pasini (1960) Ability to cause contracture of the frog rectus is greatly reduced by replacement of the 3 hydroxyl group by a 2- or 4-hydroxyl group, or by a 3-methyl, 3-methoxyl, or 3-chloro group The 3 4-dihydroxy compound (V 10) is also less active than leptodactyline (equipotent molar ratio, 5) A hydroxyl group placed β- to the onium group (V 11) reduces activity very greatly (ratio 200), but the β-keto compound, *m*-hydroxyphenacyltrimethylammonium, (V 12) is not so feeble (ratio, 9)

V 10   V 11   V 12
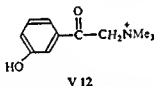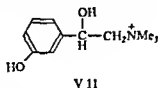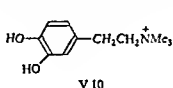
Decamethonium and other decamethylene bis-onium salts

The knowledge that simple mono-onium salts were neuromuscular blocking agents and that (+) tubocurarine, a potent blocking agent (see below), was a bis-onium salt (King, 1935, 1936), stimulated interest in bis onium salts and led to the discovery of high blocking activity in the substance Decamethonium (V 13, Barlow and Ing, 1948, Paton and Zaimis, 1948, 1949, Castillo, Phillips, and De Beer, 1949, this substance was first prepared by

$$R_3\overset{+}{N}-(CH_2)_{10}-\overset{+}{N}R_3$$
$$R_3\overset{+}{N} = Me_3\overset{+}{N}, \text{ Decamethonium, V 13,}$$
$$= Me_2Et\overset{+}{N}, \text{ V 14,}$$
$$= MeEt_2\overset{+}{N}, \text{ V 15,}$$
$$= Et_3\overset{+}{N}, \text{ V 16}$$
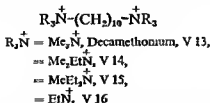
Von Braun, 1912) The action of this compound, however, is not like that of (+) tubocurarine The substance acts like acetylcholine in producing contracture of the frog rectus (Paton and Zaimis, 1949) or the chick biventer (Ginsborg and Warriner, 1960), it depolarizes the end plate of the cat's gracilis muscle (Burns and Paton, 1951) and desensitizes the end plate of the tenuissimus muscle of the cat to acetylcholine applied electrophoretically (Thesleff, 1958)

Ability to cause contracture of the frog rectus depends upon chain length

and is not actually maximal in Decamethonium (Table V 6) Replacement of
the methyl groups attached to the quaternary nitrogen atom by ethyl groups
leads to a marked decrease in activity on the frog rectus and also in ability to
depolarize the frog sartorius (Ariens and De Groot, 1954, Thesleff, 1955)
The decrease is particularly marked in going from the *bis*-ethyldimethyl
compound (V 14), in which one pair of methyl groups has been exchanged,
to the *bis*-methyldiethyl compound (V 15), *in which only one pair is left*
For the production of contracture of the chick biventer, the equipotent
molar ratios relative to Decamethonium were 3 2, 28, and 350 for the com-
pounds V 14, V 15, and V 16 respectively (Barlow and Zoller, 1962) Neuro-
muscular blocking activity (Table V 7), however, which decreased markedly
when one pair of methyl groups was replaced by ethyl groups, increased with
further ethylation (Barlow, Roberts, and Reid, 1953, Thesleff and Unna,
1954) On the rabbit quadriceps the equipotent molar ratios for blocking
activity relative to Decamethonium were 15 for the *bis*-ethyldimethylam-
monium compound, 10 for the *bis* methyldiethyl, and 4 for the *bis* triethyl
(V 16)

Replacement of quaternary ammonium by quaternary phosphonium also
decreased activity Ginzel, Klupp, Kraupp, and Werner (1953) found that
decamethylene *bis* trimethylphosphonium actually antagonized the action of
acetylcholine on the frog rectus The equipotent molar ratio relative to
Decamethonium for blocking activity on the cat gastrocnemius was 5
Decamethylene *bis*-dimethylsulphonium (V 17) was even less active Walker

$$Me_3\overset{+}{S}-(CH_2)_{10}-\overset{+}{S}Me_2$$
$$V\ 17$$
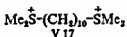
(1950) obtained an equipotent molar ratio relative to Decamethonium in the
head drop test of 15 Both the phosphonium and sulphonium analogues of
Decamethonium, like the ethyl analogues (V 15 and 16), should really be
considered as antagonists of acetylcholine

The replacement of one quaternary ammonium group in Decamethonium
by a primary amino group also reduces activity ω-Aminoundecyltrimethyl-
ammonium (V 18), which will be a *bis* onium salt with the amino group

$$Me_3\overset{+}{N}-(CH_2)_{11}-\overset{+}{N}H_3$$
$$V\ 18$$

ionized at physiological pH, had an equipotent molar ratio relative to
acetylcholine, on the frog rectus treated with eserine, of 56 (Barlow, Blaschko,
Himms, and Trendelenburg 1955) It was about as active as Decamethonium
when tested for blocking properties on the rat diaphragm, but much less
active on the cat gastrocnemius The decamethylene compound was more
active than the undeca- or dodeca methylene, and the equipotent molar ratio
was of the order of 10 relative to Decamethonium, although the shorter time
course of the block produced by the compound with the primary amino
group made comparison difficult The block was preceded by an increase in
the size of the twitch response and was therefore considered to be an acetyl-

TABLE V 6

*Activity of bis onium Salts on the Frog Rectus Equipotent Molar Ratios
Relative to Acetylcholine*

$$Me_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}Me_3$$

n =	6	7	8	9	10	11	12	18
ratio =	5 900*	420	78	15	7·4	5·5	5·0	320

* Partial agonist and antagonized acetylcholine Decamethonium is the compound where
n = 10

Paton and Zaimis (1949)

$$Me_3\overset{+}{N}CH_2CH_2-O-CO-(CH_2)_n-CO-O-CH_2CH_2\overset{+}{N}Me_3$$

n =	1	2	3	4	5	8
ratio =	—	7 0	0 84	0 21	0 21	0 11
		(20)		(3 6)		

Suxamethonium is the compound where n = 2

Bovet Bovet Nitti Guarino Longa and Marotta (1949) the values in parentheses were
obtained by *Ariens and Van Rossum (1957)*

$$CH_3CO-O-CH_2CH_2\overset{Me}{\underset{Me}{\overset{|}{\underset{|}{N}}}}-(CH_2)_n-\overset{Me}{\underset{Me}{\overset{|}{\underset{|}{N}}}}CH_2CH_2-O-CO-CH_3$$

n	9	10	11	12
ratio =	4 300	13	160	140

Barlow (1955)

$$Me_3\overset{+}{N}CH_2CH_2-O-CO-NH-(CH_2)_n-NH-CO-O-CH_2CH_2\overset{+}{N}Me_3$$

n	0	4	6	8	10
ratio =	2 3	6 0	0 72	0 55	0 13

Carbolonium is the compound with n = 6

Klupp Kraupp Stormann and Stumpf (1953) Erspamer and Glässer (1957) obtained ratios
of 8 9 for murexine 2 8 to 3 2 for Suxamethonium and 8 8 for Decamethonium

$$R_3\overset{+}{N}-(CH_2)_{10}-\overset{+}{N}R_3$$
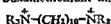
R₃ =	Equipotent molar ratio relative to acetylcholine	Intrinsic activity (page 7)
Me₃	36	0 8
Me₂Et	72	0 3
MeEt₂	inactive	—

Ariens and De Groot (1954)

$$R_3\overset{+}{N}CH_2CH_2-O-CO-(CH_2)_n-CO-O-CH_2CH_2\overset{+}{N}R_3$$

	R₃ =			
n = 2	Me₃ (Suxamethonium)	20*	1	
	Me₂Et	84	0 9	
	MeEt₂	190 (1 100*)	0 05	
n = 4	Me₃	3·6 (2*)	1	
	Me₂Et	3 8	0 9	
	MeEt₂	190 (2*)	0 4	

The figures marked with an asterisk were obtained by *Ariens and De Groot (1954)* the
remaining figures in this section of the table were obtained by *Ariens and Van Rossum
(1954)* who used Suxamethonium as standard and have been calculated on the assumption
that the value for Suxamethonium is 20 as was found by *Ariens and De Groot*

choline-like desensitization It appears, therefore, that although increase in the size of the onium group, as in the phosphonium or sulphonium analogues, destroys acetylcholine-like activity, the replacement of one quaternary ammonium group by a primary amino group merely reduces it to something comparable with that of a mono-onium salt (Table V.I)

The replacement of one pair of methyl groups in Decamethonium by an amino group, as in decamethylene bis-NN dimethylhydrazinium (V.19), also

$$\text{Me}-\overset{+}{\underset{\underset{\text{Me}}{|}}{\text{N}}}-(\text{CH}_2)_{10}-\overset{+}{\underset{\underset{\text{Me}}{|}}{\text{N}}}-\text{Me}$$

V 19
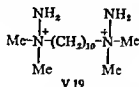
leads to a marked decline in activity Schueler and Hanna (1952) give a figure for the head-drop dose in rabbits which indicates an equipotent molar ratio relative to Decamethonium of somewhere around 30

It seems to be very difficult to alter the structure of Decamethonium without seriously reducing its acetylcholine-like properties A polymeric form of Decamethonium (V 20, n approximately = 37), which might be expected to

$$\text{Me}_3\overset{+}{\text{N}}(\text{CH}_2)_{10}-\left[\overset{+}{\underset{\underset{\text{Me}}{|}}{\text{N}}}-(\text{CH}_2)_{10}\right]_n-\overset{+}{\underset{\underset{\text{Me}}{|}}{\text{N}}}-(\text{CH}_2)_{10}-\overset{+}{\text{N}}\text{Me}_3$$

V.20
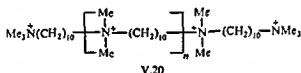
act like Decamethonium itself, was tested on the sciatic gastrocnemius of the chick by Schueler and Keasling (1956) It was less active (equipotent molar ratio relative to Decamethonium, 4), although its effects were long lasting, and it did not cause contracture A number of tris-onium salts, tested by Kensler, Zirkle, Matallana, and Condouris (1954), were also not very active Even the cyclic compound, Cyclomethone (V 21), which possesses the

Cyclomethone, V.2t

V.22
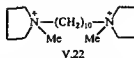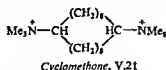
bis trimethylammonium groups of Decamethonium, appears to be predominantly curare-like, being reversed by Neostigmine (Kerp, 1957, Votava and Metysova, 1959) It was slightly more active than (+) tubocurarine-chloride in the rabbit head drop test

Decamethylene bis-N methylpyrrolidinium (V 22), however, in which two pairs of methyl groups in Decamethonium are replaced by pyrrolidine rings, does cause contracture of the frog rectus although it is not particularly potent the equipotent molar ratio relative to acetylcholine appears to be greater than 40 (Mason and Wien, 1955) On the cat gastrocnemius it is thought to block by an action like Decamethonium and is about as active as

(+)-tubocurarine chloride so the equipotent molar ratio relative to Decamethonium should be of the order of 10

Analogues in Which the Chain is Altered

Part of the polymethylene chain in Decamethonium may be replaced by a *p* linked benzene ring Wien and Mason (1953) found neuromuscular blocking activity in this type of compound to be maximal in *p*-phenylhexamethylene bis-trimethylammonium (V 23) which was about as active as (+) tubocurarine chloride on the cat gastrocnemius preparation, and therefore presumably has an equipotent molar ratio relative to Decamethonium of about 10 The substance caused contracture of the frog rectus and the block was unaffected by eserine The analogous *bis*-triethylammonium compound had similar neuromuscular blocking activity, but did not cause contracture, and the effects were reversed by eserine The activity of Decamethonium is reduced to about the same extent by the introduction of triple bonds in the 2-3 and 8 9 positions (V 24, Marszak, Jacob, and Guermont, 1953)

$$Me_3\overset{+}{N}-CH_2C\equiv C-(CH_2)_4-C\equiv C-CH_2\overset{+}{N}Me_3$$
V.24
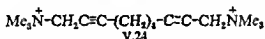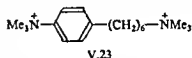
The replacement of part of the chain by a saturated ring seems to conserve the activity of Decamethonium better than replacement by an unsaturated ring Randall (1952) has described the high neuromuscular blocking activity of hexamethylene-*bis*-4-(N-dimethyl)piperidinium (V 25) On the cat tibialis

V 25
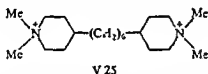
preparation this was eight times as active as (+)-tubocurarine chloride, in the rabbit head-drop test it was half as active, and in the mouse inclined screen test it was one-eighth, its effects were not antagonized by *Edrophonium* (an anticholinesterase, see page 263) These results indicate an action like Decamethonium and an equipotent molar ratio of about 1 When one methyl group at each end of the molecule was replaced by *p* nitrobenzyl, the compounds were antagonized by *Edrophonium* and appeared to be curare-like Randall thought that this change in activity might be related to the introduction of the *p* nitrobenzyl group into the molecule and studied the effects of similar substitution into Decamethonium The most active member of this series of polymethylene *bis p* nitrobenzyldimethylammonium salts was actually the nonamethylene compound (V 26) (equipotent molar ratio relative to Decamethonium approximately 8 on the cat tibialis), but the
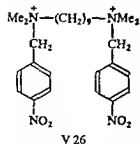
V 26

effects were reversed by *Edrophonium* It may be questioned whether the change in the character of the block really depends on the introduction of the *p*-nitrobenzyl group or on the removal of one of the methyl groups attached to the onium atom

Compounds in which one of the methylene groups of Decamethonium is replaced by an ether oxygen atom (V 27) were studied by Levis, Preat, and

$$Me_3\overset{+}{N}-(CH_2)_n-O-(CH_2)_m-\overset{+}{N}Me_3$$
V.27.

$n = m = 5$, *Brevatonal*, V.28,
$n = 4, m = 5$, V.29
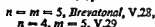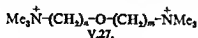
Dauby (1953) In this series activity was maximal when $n = 5$ (V 28 *Brevatonal*) The block produced by this compound was relatively short lasting, and the equipotent molar ratio relative to Decamethonium was 1 5 on the rabbit gastrocnemius and 5 in the rabbit head drop test This compound caused contracture of the frog rectus preparation, unlike its *bis* trimethyl analogue The equipotent molar ratio for the compound (V 29) with the same chain length as Decamethonium was 3 to 16 on the cat gastrocnemius (Marszak, Jacob, and Guermont, 1953)

The much longer compound, *Prestonal* (V 30), is of interest because it

$$Me-\overset{Me}{\underset{CH_2}{\overset{+}{N}}}-CH_2.CH_2-O-(CH_2)_{10}-O-CH_2.CH_2-\overset{Me}{\underset{CH_2}{\overset{+}{N}}}-Me$$

with lower groups: C=O / O / nPr
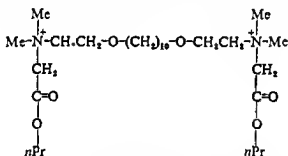
Prestonal, V.30

appears to block by desensitization (Frey, 1956, Baker, Foldes, Birch and D Souza, 1956) The equipotent molar ratio compared with succinylcholine (see below) in man is 1 4 (Frey, 1956), which suggests a ratio to Decamethonium of 5 to 10 Lengthening the chain in the Decamethonium molecule appears either to decrease acetylcholine-like blocking activity or to increase curare like activity, the tridecamethylene compound (V 31) for example, appears to

$$Me_3\overset{+}{N}-(CH_2)_{13}-\overset{+}{N}Me_3$$
V.31

act in some way by both mechanisms producing what has been called a 'mixed block' (Jewell and Zaimis 1954) Further, one of the methyl groups in each of the onium ends is, in *Prestonal* replaced by a larger group (prop oxycarbonylmethyl) For these two reasons therefore, it is surprising that *Prestonal* should block to any extent by an acetylcholine like desensitization

Derivatives in which the link is through a sulphur atom, S thuanonane and

5 thiaundecane *bis* trimethylammonium salts (V 32) have been prepared by Andrews, Bergel, and Morrison (1953), and appear to produce neuromuscular

$$Me_2\overset{+}{N}-(CH_2)_n-S-(CH_2)_n-\overset{+}{N}Me_3$$
$$n = 4 \text{ and } 5, \text{ V } 32$$
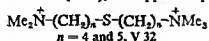
block by an action similar to that of Decamethonium The dithia compound, 5 6-dithiadecamethylene *bis* trimethylammonium (V 33), produces

$$Me_3\overset{+}{N}-(CH_2)_4-S-S-(CH_2)_4-\overset{+}{N}Me_3$$
$$\textbf{V 33}$$
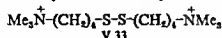
effects which are quantitatively and qualitatively very similar to those produced by Decamethonium (Hunter, 1953)

Compounds containing more than one ether link in the decamethylene chain are not particularly active (Levis, Preat, and Dauby, 1953), although

$$Me_3\overset{+}{N}-(CH_2)_2-S-(CH_2)_2-S-(CH_2)_2\overset{+}{N}Me_3$$
$$\textbf{V 34}$$
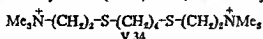
3 8-dithiadecamethylene *bis* trimethylammonium (V 34) was almost as active as Decamethonium in mice (equipotent molar ratio, 1 3, Taylor, 1952)

TABLE V 7

Neuromuscular Blocking Activity

$$Me_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}Me_3$$
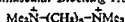
	$n =$	7	8	9	10	11	12
Equipment molar ratio relative to Decamethonium (cat tibialis)		100	5 5	12	10	20	31

Paton and Zaimis (1949)

$$Me_2\overset{+}{N}CH_2CH_2\cdot O-OC-(CH_2)_n-CO-O-CH_2CH_2\overset{+}{N}Me_3$$

	$n =$	0	1	2	3	4	5	8
Equipotent molar ratio relative to Suxamethonium (rabbit head-drop)		370	10	10	25	25	15	25

Bovet, Bovet Nitti, Guarino, Longo, and Fusco (1951)

$$Me_3\overset{+}{N}CH_2CH_2-O-OC-NH-(CH_2)_n-NH-CO-O-CH_2CH_2\overset{+}{N}Me_3$$
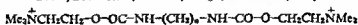
	$n =$	0	1	2	3	4	5	6	7	8	10
Equipotent molar ratio relative to Carbolonium (rabbit head-drop)		100	20	34	24	9 1	4 2	1 0	0 85	—	2 6
(cat gastrocnemius)		—	—	7 4	—	1 0	—	1 4	1 9		

Cheymol et al (1954)

Klupp, Kraupp, Stormann, and Stumpf (1954)

Bis-onium Salts Which Are Esters

The replacement of part of the decamethylene chain by an ester group does not seem to be detrimental to activity The compound (V 35) analogous

$$Me_3\overset{+}{N}-CH_2CH_2-O-\underset{\underset{O}{\|}}{C}-(CH_2)_5-\overset{+}{N}Me_3$$
$$\textbf{V 35}$$
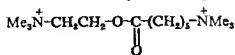
to Nonamethonium has a head-drop dose in the rabbit about twice that of Decamethonium (Bovet, Bovet Nitti, Guarino, Long, and Fusco, 1951)

Compounds containing more than one ester group are particularly interesting The compound of greatest interest as a blocking agent is succinylcholine (Suxamethonium, V 36, Table V 7, Bovet *et al* , 1949, Phillips, 1949,

$$Me_3\overset{+}{N}-CH_2CH_2-O-\overset{\overset{O}{\|}}{C}-(CH_2)_2-\overset{\overset{O}{\|}}{C}-O-CH_2CH_2\overset{+}{N}Me_3$$
Suxamethonium, V 36
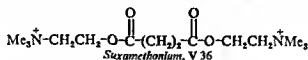
Walker, 1950) This substance was actually described by Hunt and Taveau (1906), but was unfortunately tested in a 'curarized' animal and its neuromuscular blocking activity went unnoticed for over 40 years This compound is more active than Decamethonium when tested on the frog rectus but is, in its turn, less active than the higher members of the series (Table V 6) The neuromuscular blocking effects of succinylcholine in intact animals are very short lived because the compound is rapidly hydrolysed by the cholinesterases of plasma (Chapter VIII) to the much less active half ester (V 37) It is a most useful clinical tool

$$Me_3\overset{+}{N}-CH_2CH_2O-\overset{\overset{O}{\|}}{C}-(CH_2)_2-\overset{\overset{O}{\|}}{C}-OH$$
V 37
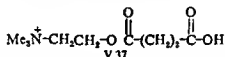
The effects of substitution in the onium groups of this molecule are very similar to those in Decamethonium The replacement of one methyl group by ethyl in each onium group lowers activity slightly, but replacement of a second pair lowers it markedly (Ariëns and van Rossum, 1957) The sulphonium analogue of succinylcholine, however, causes contracture of the frog rectus (the equipotent molar ratio relative to acetylcholine appears to be about 200) The head drop dose in the rabbit is about ten times that of succinyl choline When injected intra arterially close to the tibialis muscle of the cat it causes contracture, and its subsequent blocking action can be reversed by a suitable dose of (+)-tubocurarine chloride (Della Bella, Villani, and Zuanazzi, 1956)

Similar esters of choline with polymethylene *bis*-carbamic acids (V 38)

$$Me_3\overset{+}{N}-CH_2CH_2-O-\overset{\overset{O}{\|}}{C}-NH-(CH_2)_n-NH-\overset{\overset{O}{\|}}{C}-O-CH_2CH_2\overset{+}{N}Me_3$$
V 38
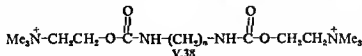
have been studied by Klupp, Kraupp, Stormann, and Stumpf (1954) and Cheymol, Delaby, Chabrier, Najer, and Bourillet (1954) Both acetylcholine-like and neuromuscular blocking activity here appears to be associated with a much longer chain length between the onium groups, but neuromuscular blocking activity reaches a maximum in the heptamethylene *bis* carbamyl ester, whereas activity in the frog rectus is greatest in the decamethylene *bis*-carbamyl ester, the highest member of the series to be studied These compounds possess appreciable anticholinesterase activity

When two acetylcholine molecules are attached by a chain through their onium groups, as in polymethylene *bis* acetoxyethyl dimethylammonium salts (V 39), the activity on the frog rectus is more sharply sensitive to alterations in chain length The decamethylene compound (equipotent molar ratio relative to acetylcholine, 13) is 100 times as active as the nonamethylene compound and ten times as active as the undecamethylene and dodecamethylene compounds Table V 6 (Barlow, 1955) Its blocking properties have not been studied to any extent

$$CH_3C-O-CH_2CH_2-\overset{+}{\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{N}}}-(CH_2)_n-\overset{+}{\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{N}}}-CH_2CH_2-O \ C-CH_3$$

V 39
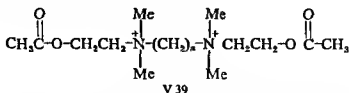
Value of Information Obtained From the Experiments With the Frog Rectus

The aim of this section is to review what is known about the relationships between structure and ability to act like acetylcholine at the neuromuscular junction In default of *detailed information* about the ability of compounds to produce electrical changes at the end plate similar to those produced by acetylcholine, it is necessary to fall back on some *more simply observed* action, such as the production of contracture of slow fibres The value of results obtained with the frog rectus, however, is clearly limited Estimates of equipotent molar ratios vary very greatly, apparently by as much as a factor of 10 This could be attributed to the different amounts of cholinesterase present in different preparations and to destruction of some drugs, but not others, by this enzyme If all the experiments were performed in the presence of an anticholinesterase, or with reference to a standard not destroyed by cholinesterase (such as tetramethylammonium) more consistent figures might be obtained Within any one set of experiments however, the conditions are not so likely to fluctuate greatly, and the results may give some indication of how activity varies with structure within a series Of the compounds discussed, the most active seem to be propionyl and valeryl choline, dihydromurexine and β (α-pyridyl) propionylcholine (V 2), the very long compounds sebacylcholine and decamethylene *bis* carbamylcholine and possibly leptodactyline

Relationships Between Structure and Ability to Cause Contracture of Slow Fibres

In view of the classification of both the neuromuscular junction and autonomic ganglia as sites of the nicotine like actions of acetylcholine, it seems reasonable to expect that the relationships between structure and activity at the two sites may, in general, be somewhat similar Hey (1952) suggested that nicotine like stimulant activity in ganglia is associated with the presence in a molecule of a cationic head and a partial positive charge about the same distance away from it as is the ether oxygen in acetylcholine (Chapter VI) It seems possible that these ideas can be extended to the neuromuscular junction

The importance of the cationic head for activity in causing contracture and the decrease in activity with increasing size of the head are seen in Table V 2 Ing (1949) suggested that the results were compatible with the binding of the onium group at a surface, two such possibilities are illustrated in Fig V 8 This is consistent with the observation that replacement of one methyl group in the head by the larger ethyl group lowers activity slightly, presumably because the onium group can now fit only in one way, with the ethyl group away from the receptor surface Replacement of two methyl groups by ethyl, however, should greatly reduce the ability to fit the receptor and virtually destroys activity This interpretation, however, is open to criticism because of the findings of Barlow, Scott and Stephenson (1963)
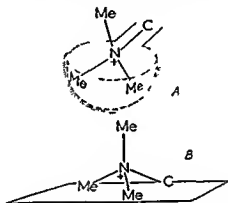
FIG V 8   *Adsorption of acetylcholine A At a hemispherical cavity B At a planar surface*

The activity of acetyl α methylcholine (equipotent molar ratio, 2) suggests that the α methyl group spoils the fit of the molecules to the receptor but that, like the ethyldimethyl ammonium compounds, it can still combine with the receptor by a suitable rotation of the C—C or C—N bonds in the choline residue Substitution of a methyl group on the β-carbon atom, however, is highly detrimental to activity This substitution is unlikely to affect the attachment of the onium group, and the effect on activity is greater than would be expected if the effect were simply on the attachment of the choline part of the molecule The results strongly suggest that this β methyl group interferes drastically with the attachment of the molecule at some nearby point, possibly at the seat of the partial positive charge postulated by Hey (1952) It could do this either by steric interference or by its electronic effects in reducing the size of the partial positive charge

In acetylcholine the most likely atom in this region to possess a positive charge of any magnitude is the carbonyl carbon atom If the effect of the β-methyl group were exclusively electronic, it might be expected that addition of a methyl group to the acetyl portion should be even more effective In fact, propionylcholine has the same order of activity as acetylcholine, so it must be concluded that the effect of the β methyl group is steric rather than

electronic Possibly it restricts access to the carbonyl carbon atom of the ester link

The importance of the carbonyl group in acetylcholine is clearly shown by comparing the high activity of ketones, such as 3- and 4-keto *n*-pentyltrimethylammonium and 3 keto *n* butyltrimethylammonium, with the feeble activity of the analogous ethers Only in phenylethers of choline, in which the ether oxygen atom may have a considerable positive charge, is there much activity on the frog rectus (e g the *m* bromophenyl but not, surprisingly, the *p* chlorophenyl ether) The relationships between the structure and activity of the ketones can be explained if it is assumed that there are two factors involved the partial positive charge must be suitably placed somewhere around the 3- or 4-positions relative to the onium group, and secondly, the activation of the carbonyl group must be appropriate The ethyl ketones might be less active than the methyl ketones because of the greater inductive effect of the ethyl group de activating the carbonyl group (the difference between the inductive effects of ethyl and *n* propyl is less than that between methyl and ethyl)

The activity of the simple aliphatic esters of choline appears to contradict these ideas because the propionyl and butyryl esters are more active than acetylcholine on the frog rectus When tested in the presence of eserine, however, propionylcholine is less active than acetylcholine, and it seems that the apparently greater activity on the frog rectus should be ascribed only to greater stability

High activity on the frog rectus is shown by dimethylacryloylcholine, murexine, and α pyridylacryloylcholine (Table V 3) but whereas the activity of the two latter is increased by reduction, that of the former is slightly decreased Matters are again complicated by the relative stability or instability of the compounds, but the high activity of the saturated compounds suggests that the electronic effects of the ring on the carbonyl carbon atom of the ester group are not important Hydrogenation of the double bond destroys the conjugation between the ring and the carbonyl group The ring itself must nevertheless be important, and it is conceivable that there is an interaction between partially charged atoms in either the iminazole or pyridine rings and partially charged groups on the receptor The double bond in dimethyl acryloylcholine is also polarized and might contribute to the stability of the complex with the receptor, this would largely be destroyed by reduction of the double bond

The high activity of the very long *bis* compounds is remarkable, but in both series, the choline esters of polymethylene dicarboxylic acids and those of polymethylene *bis* carbamic acids, activity increases only gradually with chain length This suggests that each additional methylene group contributes a small increment to the adsorbability through van der Waal's forces (cf page 211), the total contribution from a large number of these being very considerable The function of the second onium group, then, would be to limit properties such as surface activity rather than to serve as an additional point of attachment

E

In nicotine itself three parts of the molecule appear to be important, because the natural (−) isomer, which has the S-configuration (Hudson and Neuberger, 1950), appears to be more active than the (+) isomer (review by Barlow, 1964) From the high activity of compounds related to nicotine which are not optically active, however, it seems likely that this third group, additional to the cationic head and the pyridine ring, does not so much endow the (−) isomer with high activity as destroy activity in the (+) isomer, possibly by reducing its affinity for the receptors

TABLE V 8

Comparison of the Activity of Some Neuromuscular Blocking Agents

	Man anaesthesia					Rabbit head-drop	
	Dose mM/Kg		Equipotent molar ratio		Duration of effect (min)	Dose mM/Kg	Equipotent molar ratio
	(*)	(†)	(*)	(†)	(*)		
(+)-Tubocurarine chloride	430	140–360	5 7	0 74–4 5	40	216	0 78–1 1
(+)-Tubocurarine dimethyl ether iodide	110	—	1 5	—	40	—	—
Gallamine triethiodide	1 120	1,300–2,200	15	6 8 27	15–30	505	1 8–2 6
Benzoquinonium	320	—	4 3	—	15 20	—	—
Laudexium	465	—	6 2	—	>40	—	—
Carbolonium‡	90–130	—	1 2–1 7	—	30–60	—	—
Murexine	—	3 900–4,700	—	860–1 700	—	2,600	9 5–13
Decamethonium	75	80–190	1	1	10–20	195–275	—
Suxamethonium	250–500	860–1 700	3 3 67	4 5–31	1 3	340–430	1 2–2 2

The equipotent molar ratio is calculated relative to Decamethonium from * *Robson and Keele* (1956) † *Erspamer and Glasser* (1957) ‡ *Brucke and Reis* (1954)

In this discussion it has not been possible to separate the effects of chemical structure on affinity from effects on efficacy There is no reason to suppose that increased affinity must necessarily lead to increased activity Further, it is by no means clear how far ability to act like acetylcholine in causing contracture is related to ability to block transmission by acting like an excess of acetylcholine It might be argued, by comparing the activity of the dicarboxylic esters of choline on the frog rectus and in the rabbit head-drop tests, that there was no connexion between the two, because Suxamethonium was the most active in the latter and relatively inactive in the former The rate of hydrolysis of the esters by the cholinesterases present in serum, however, increases with chain length (Bovet Nitti, 1949), and it is possible that the

lower activity of the long-chain compounds in the head-drop test may he due to their low stability in the animal In the polymethylene *bis* carbamylcholine series, activity in both the tests increases with chain length, these compounds are actually inhibitors of cholinesterases While, in general, the ability to cause contracture and the ability to cause a block by desensitization appear to he associated, it cannot he definitely asserted that the ability to desensitize is directly related to the ability to depolarize Some substances (e g Decamethonium) certainly appear to have greater desensitizing activity than would he expected from their ability to cause contracture and contrariwise the ability of acetylcholine to desensitize is less than would he expected from its ability to depolarize, perhaps because of the limits to its effects imposed by cholinesterase Thus depolarization and contracture may depend upon adsorption and the production of 'stimulus', hut desensitization may he related to slowness in desorption and persistence at the receptor (see page 17) It is certainly noticeable that the contracture produced hy compounds like Decamethonium on the frog rectus has a different time course from that produced by acetylcholine, it is much slower in onset and takes a longer time to reach a maximum The relative neuromuscular blocking activity of a number of agents in man is shown in Tahle V 8

ANTAGONISTS

Compounds Related to Tubocurarine

The chemistry of the alkaloids of tube curare has been worked out in some detail The first of these to he obtained pure was (+)-tuhocurarine chloride (King, 1935) In the early clinical use of neuromuscular blocking agents crude extracts of curare alkaloids were employed These were standardized hy bioassay (see, for instance, the British Pharmacopoeia for 1953), (+)-tuhocurarine chloride being a suitable standard for comparison As the pure alkaloid has become more available, and synthetic substitutes have been developed, the use of crude extracts has ceased

King (1935, 1936) showed that (+) tuhocurarine chloride was a *bis*-tetrahydroisoquinoline derivative, containing two quaternary nitrogen atoms and two free phenolic groups, and eventually established the position of the latter and the structural formula (V 40, King, 1948)

The chemistry of compounds related to tubocurarine is reviewed by Wintersteiner (1959) and the alkaloid has been synthesized by Voronin, Tolkachev, and Preobrazhenskii (1958)

R = Me, R' = H, *Tubo-curarine*, V 40,

R = H, R = Me *Chondocurarine* V 41

There are two asymmetric centres and all the four isomers, (+)- and (−)-tuhocurarine and (+)- and (−)-N-dimethylcurine are known (curine is also referred to as hebeerine or chondodendrine) The isomer chondocurarine differs only in the relative position of a phenolic and a methoxyl group
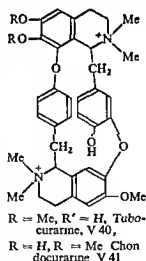
ability of the compound to antagonize the contracture of the frog rectus produced by acetylcholine The pKa values were 8 1 and 9 1, and Kalow concluded that the former was for the ionization of the simple phenolic group and the latter for the ionization of the hydroxyl group in the 7 position of the tetrahydroisoquinoline ring At pH 7 4, 82 per cent of the (+) tubocurarine is present as the simple bis onium salt, but 16 per cent has a dissociated phenolic group, 1½ per cent has a dissociated 7-hydroxyl group, and there are even traces of the bis-onium salt with both hydroxyl groups ionized These hydroxyl groups should be more ionized in alkaline solutions and less ionized in acid solutions, so it should be possible to deduce, from the effects of pH on activity, which is the active species It is not sufficient simply to measure the absolute activity of (+)-tubocurarine at different values of pH because the change in pH may itself alter the sensitivity of the tissue Kalow measured the activity of (+) tubocurarine relative to that of the dimethyl ether, the ionization of which will be unaffected by changes of pH On the frog rectus, in contrast to the rabbit head drop test, the dimethyl ether is not greatly different in activity from (+) tubocurarine itself At pH 6 7 it was actually less active (equipotent molar ratio, 1 5-2), but at pH 8 7 it was much more active (ratio 0 25-0 5) The relative activity of (+)-tubocurarine decreases with increasing ionization of the hydroxyl groups, and the results are consistent with the idea that it is the simple bis onium salt with undissociated hydroxyl groups which is the active species Similar studies with nicotine and nicotine monomethiodide on the rat diaphragm (Barlow and Hamilton, 1962) suggest that it is the univalent cation (V 6) which is the active species, an idea originally suggested by Taylor (1951)

Stereospecificity

Table V 9 indicates that the receptor is fairly stereospecific It appears that the configuration (−)-(+) is most favourable to activity, but (−)-(−) is also acceptable or even (+)-(+) if Wintersteiner's figures for N-dimethylcurarine are taken The low activity of N-dimethylisochondodendrine is particularly interesting Even though this bas the (+) configuration, it seems that the ether oxygen linked *meta* to the benzene ring is important to (+) tubocurarine and (+) chondocurarine It is difficult to describe exactly what difference this bridge actually makes to the way in which the molecule can become arranged, but it is likely to be considerable The two benzyl bridges between the tetrahydroisoquinoline rings cannot be in the same plane (as they appear to be in the usual drawing of the formulae), but are forced slightly inclined to each other into planes at right angles to the rest of the molecule Although the hetero-part of the tetrahydroisoquinoline rings can buckle and consequently, in theory, the attachment at the asymmetric 1-carbon atom need not be fixed equatorial or axial, the arrangement may well be limited by the inflexibility of large portions of the 18 membered ring It is not really possible to illustrate this adequately with a drawing, a molecular model should be inspected It is difficult to compute the possible distances between the two onium groups with any degree of confidence (see,

however, Battersby and Hodson, 1960, referred to on page 132), but it would appear that these do not vary as greatly as do the distances between the simple phenolic hydroxyl group and either of the onium groups It is conceivable that this phenolic group provides a third point of attachment to the receptor (there must be at least a three-point attachment to account for the stereospecificity), but consideration should also be given to the ether oxygen atom between the two aromatic rings which should carry an appreciable partial positive charge

Development of Synthetic Neuromuscular Blocking Agents

Among the compounds immediately related to (+) tubocurarine the quaternary salts are always more active than the tertiary bases. The neuromuscular blocking activity of quaternary salts was demonstrated long ago by Crum Brown and Fraser (1869), who repeated Bernard's experiments on the frog with the methiodides of strychnine, brucine, thebaine, codeine, morphine, atropine, and conine. Although the pharmacological properties of these alkaloids are exceedingly diverse, the quaternary salts all produced paralysis Tetra-alkyl ammonium, phosphonium, arsonium, stibonium, trimethylsulphonium, and even iodonium salts were subsequently examined (review by Ing, 1936), and it became recognized that neuromuscular blocking properties are characteristic of onium salts as a class It will now be appreciated that this generalization requires qualification to exclude substances, such as Decamethonium, which act like acetylcholine and cannot be regarded as blocking transmission by exactly the same mechanism as (+)-tubocurarine chloride. Nevertheless, the observation appears to be generally true for onium salts with only two, or fewer, methyl groups attached to an atom in Group V of the Periodic Table

The discovery that (+)-tubocurarine is a *bis* onium salt (King, 1935, 1936) stimulated interest in compounds containing more than one quaternary group. The substances *3381 RP* and *3365 RP* (V 43, 44) which resemble parts
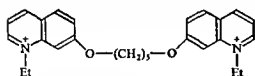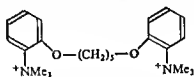
3381 RP, V 43   3365 RP, V 44

of the tubocurarine molecule were found to be only slightly less active than that compound in the rabbit head drop test (Bovet *et al*, 1946, 1947) The *tris* onium salt, Gallamine Triethiodide (V 45) had comparable activity and
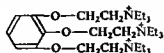
Gallamine Triethiodide, V 45

was introduced clinically (Huguenard and Boué, 1949) When tested for its ability to reduce the flexing power of the hand in man by Mushin, Wien, Mason, and Langston (1949), it was found to be less active than

(+)-tubocurarine (equipotent molar ratio, 4 2), but it is a true antagonist of acetylcholine, and its effects are reversed by an anticholinesterase such as Neostigmine (page 260)

Further developments (for reviews see Bovet, 1959, and Cavallito and Gray, 1960) were complicated by the discovery of the activity of Decamethonium and succinylcholine and the compounds can, for convenience, be divided into two groups. In the first are substances, usually of relatively simple structure, derived from desensitizing neuromuscular blocking agents and subsequently shown to block by a different mechanism. In the second are substances of more complex structure derived from (+)-tubocurarine and other alkaloids

The First Group: Relatively Simple Structures

As has already been pointed out (page 110), quite small changes in the Decamethonium molecule markedly decrease ability to act like acetylcholine at the neuromuscular junction, although the compounds are still able to block transmission by an action which is apparently like that of (+)-tubo-curarine Among the compounds of this type are the phosphonium and sulphonium analogues of Decamethonium, Cyclomethone, and the polymeric substance (V 20, see page 112). The evidence that this block is really a competition, however, is usually indirect, depending on the reversal of the block by an anticholinesterase or the absence of signs of acetylcholine like activity Ariëns and Van Rossum (1957) investigated the nature of the antagonism of the actions of acetylcholine by many derivatives of Decamethonium on the frog rectus preparation. They found that when one or more methyl group at each end was replaced by a large alkyl group (e g, n-pentyl, V 46), the antagon-

$$n\text{-Pent}-\overset{\overset{\displaystyle Me}{|}}{\underset{\underset{\displaystyle Me}{|}}{N}}-(CH_2)_{10}-\overset{\overset{\displaystyle Me}{|}}{\underset{\underset{\displaystyle Me}{|}}{N}}-n\text{-Pent}$$

V 46

ism had the characteristics of a non-competitive block It is necessary to keep in mind, therefore, the possibility that many compounds, which block transmission at the neuromuscular junction by an action different from that of acetylcholine, may not, in fact, be truly competitive antagonists of acetylcholine This may be particularly important for compounds with large aliphatic groups such as V 46, mentioned above, and polymethylene bis onium salts with very long chains The tridecamethylene homologue of Decamethonium, for instance, is less potent as a blocking agent than Decamethonium but more active in causing contracture This suggests that lengthening the chain increases ability to block by a mechanism other than desensitization In the analogous bis triethylammonium salts, for instance, considerable blocking activity is to be found among
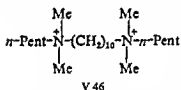
$$Et_3\overset{+}{N}-(CH_2)_n-\overset{+}{N}Et_3$$
$$n = 16 \text{ or } 17$$
V 47

compounds with a really long chain, such as hexa- and hepta decamethylene bis triethylammonium (V 47, Barlow and Zoller, 1964)

Although decamethylene bis pyridinium is not particularly active and the bis-quinolinium and iso-quinolinium analogues are only feeble (Taylor, 1951), the introduction of methoxyl groups and, more especially, the reduction of
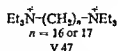
tbe heterocyclic ring greatly increases activity Decamethylene *bis* (1 2 3 4-tetrahydro 6 7-dimethoxy)-N-methylisoquinolinium (V 48) was more active than (+) tubocurarine chloride in the rabbit Taylor (1951) obtained an equipotent molar ratio of 0 35 in a test based on ability to block the righting reflex Smith, Pelikan, Maramba, and Unna (1953) obtained the ratio 0 23 by the head-drop method The compound with an additional methoxyl group in the 8 position (V 49) was more active according to Taylor's results (ratio

MeO—[structure] $\overset{+}{N}$ Me Me $\overset{+}{N}$ —OMe
MeO— —OMe
$(CH_2)_{10}$
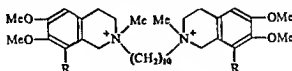
R = H, V 48,
R = MeO, V 49

0 15), but about the same (0 26) according to the results of the other group The compounds were less active on the cat tibialis (the ratio was 1 0 for the 6 7-dimethoxy compound) These compounds, like (+)-tubocurarine itself, contain two asymmetric carbon atoms in the 1-position of the reduced *iso*-quinoline ring and the tests were made with material consisting of a mixture of isomers Smith, Pelikan, Maramba, and Unna compared the activity of different fractions of some of the compounds and the results suggest some degree of stereospecificity These substances did not cause contracture of the frog rectus or chicken gastrocnemius preparations

One of the most interesting substances in this group is the substance *Benzoquinonium* (*Mytolon*, V 50, Hoppe, 1950, 1951) In the rabbit head drop

$$PhCH_2—\overset{Et}{\underset{Et}{\overset{+}{N}}}—(CH_2)_3—NH—[structure]—NH—(CH_2)_3—\overset{Et}{\underset{Et}{\overset{+}{N}}}—CH_2Ph$$
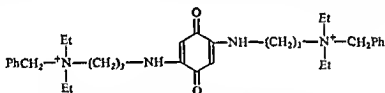
Benzoquinonium V 50

test the equipotent molar ratio for this compound was 0 2, but in the mouse inclined screen test it was 2 2 The effects of the compound were not well antagonized by *Neostigmine* and not at all by *Edrophonium* (Randall, 1951) Nevertheless, the compound does not cause contracture of the frog rectus, and the reason why anticholinesterases do not reverse its neuromuscular blocking action must be because the substance is itself a potent anticholinesterase, being about one-quarter as active as Neostigmine itself Hougs (1957) and Bowman (1958) have obtained results which confirm the idea that the compound is a competitive blocking agent

Second Group. More Complex Structures

Among the second group of compounds is the dimethyl ether of (+) tubocurarine chloride This was shown by Collier, Paris, and Woolf (1948) to be much more active than (+) tubocurarine chloride itself When tested for

ability to block the righting reflex in the mouse, rat, and rabbit, the equipotent molar ratios were found to be respectively 1 2, 0 2, and between 0 06 and 0 09 On the isolated rat diaphragm, however, the dimethyl ether was not so active, the ratio being around 0 5 Unna *et al* (1950) obtained an equipotent molar ratio of 0 37 when the compounds were compared for their ability to reduce the grip-strength in man

The variation with species of the activity of this compound relative to (+)-tubocurarine has already been mentioned in connexion with Kalow's work (1954) using the frog rectus It is certainly remarkable how big the variation is when one considers that the compounds are assumed to be acting by the same mechanism Even allowing for discrepancies which might arise from the slight differences in the pH of the test preparations, the relative activities of compounds seem to vary from species to species in a most unpredictable manner

Open chain *bis* benzylisoquinoline derivatives polymethylene *bis* laudanosinium salts (V 51) have been studied by Taylor (1952), Collier (1952), and
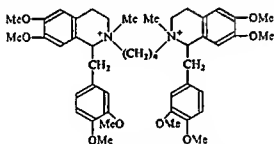
n = 10 *Laudexium* V 52

Collier and Macaulay (1952) Activity in blocking the righting reflex in rabbits is maximal in the nonamethylene member of the series (equipotent molar ratio relative to (+) tubocurarine chloride, 0 17), but the decamethylene member is almost as active (0 20) The latter (V 52 *Laudexium, Laudolissin*) was tested in man and found to be rather less active than (+)-tubocurarine chloride (equipotent molar ratio between 1 and 2), although its effects lasted relatively longer (Bodman, 1952) It did not cause contracture and its effects were reversed by Neostigmine

Tropine Derivatives

Although atropine readily blocks the sites of the muscarine like actions of acetylcholine (page 83), it only affects the neuromuscular junction when given in very high doses indeed (Kimura, Unna, and Pfeiffer, 1949) As might be expected from the results of Crum Brown and Fraser, discussed on page 125, atropine metho-salts are much more active than the base itself in blocking the neuromuscular junction Neuromuscular blocking activity is even more marked in polymethylene *bis* atropinium salts The penta- and deca methylene compounds were tested by Kimura and Unna (1950) and the

hexa- and octa methylene compounds by Eckfeld (1959) Of these, the decamethylene compound (V 53) appears to be the most active and appreciably stronger than (+) tubocurarine chloride in the rabbit head drop test (equipotent molar ratio, 0 3) The compound was relatively less active when tested on the dog gastrocnemius or rat diaphragm preparations, the block was antagonized by Neostigmine

$$R = Ph\text{-}CH\text{-}CO \quad V 53$$
$$\qquad \ \ CH_2OH$$
$$\ \ = PhCH_2CO \quad V 56$$
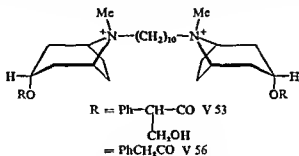
A similar compound, 1 4-xylylene *bis* atropinium (V 54), was about as active as (+) tubocurarine chloride (Issekutz, 1952), the equipotent molar ratio in the rabbit head drop test was 0 8 (review by Gyermek and Nador, 1957) The tropic acid part of the molecule is important, however, as well as the polymethylene chain 1 4-Xylylene *bis* benzoyl α tropinium (V 55, which has the same configuration at the 3 position as atropine, i e the ester link is

$$R = Ph\text{-}CH\text{-}CO \quad V 54$$
$$\qquad \ \ CH_2OH$$
$$\ \ = PhCO \quad V 55$$
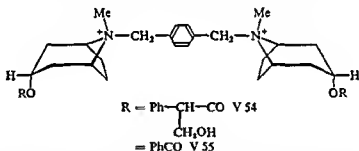
trans or axial, see page 214) was more active (equipotent molar ratio relative to (+)-tubocurarine chloride, 0 6) Decamethylene *bis* phenylacetoxytropinium (V 56) on the other hand, was less active than the corresponding tropic ester (equipotent molar ratio relative to (+) tubocurarine chloride 1 0 on the cat gastrocnemius and 1 1 in the rabbit head drop test, Haining, Johnston and Smith, 1960) The succinic ester, *m* brombenzyl succinyl α tropinium (V 57) in which the two tropine fragments are linked at the 3 position instead

V 57
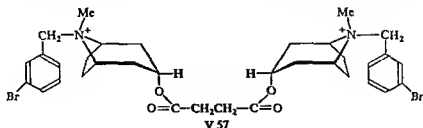
of through the nitrogen atom, had an equipotent molar ratio of 0 3 relative to (+)-tubocurarine chloride in the rabbit head-drop test, but its effects were much more transient It was relatively less active in the cat and dog and its effects were reversed by *Edrophonium* Haining, Johnston, and Smith (1960) also studied a rather similar series in which the bridge was between the two nitrogen atoms The most active compounds were those in which the link was 4 8-dioxo-3 9-dioxaundecamethylene (V 58) and 5 8 dioxo-4 9-dioxadodecamethylene, which had equipotent molar ratios relative to (+)-tubocurarine chloride of 1 1 and 0 9 respectively on the cat gastrocnemius and 0 7 and 1 0 in the rabbit head-drop test The effects of these compounds resembled those of *Suxamethonium* in being short-lived, but the

$$\text{Me} \atop \text{H} - \overset{}{\underset{RO}{\diagdown}} \text{N-(CH}_2)_3\text{-O-C-C-(CH}_2)_3\text{-C-O-(CH}_2)_3\text{-N} \overset{\text{Me}}{\underset{OR}{\diagup}} - \text{H}$$
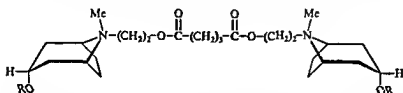
R = PhCH₂CO, V 58

compounds did not cause contracture of the frog rectus In two instances it was possible to test pairs of epimers in which the configuration of the groups about the quaternary nitrogen atom was different In atropine the methyl group tends to be axial and alkylation introduces the fourth substituent equatorially There was little difference between the activity of the two epimeric forms in these instances Nador (1960) reported little difference between epimers where the substituents were methyl and butyl, but considerable differences when one of the groups was aralkyl He found a 40 fold difference between the two forms of 1 4-xylylene *bis* benoyloxy-α tropinium bromide

The position of the ester group (axial, α-, as in tropine, or equatorial, β- as in pseudo tropine) does not appear to be very important, although there are only a few instances in which both the α tropine and β-tropine derivatives have been compared (the compounds so far discussed are all α-linked) Of the two isomeric forms of 1 6-hexamethylene *bis* benzoyltropinium bromide, however, the β- was more active than the α- (the equipotent molar ratio of the β-compound relative to (+)-tubocurarine chloride in the rabbit head drop test was 0 3)

β-Erythroïdine

This alkaloid (V 59, Boekelheide, Grundon, and Weinstock, 1952) is of interest because it is one of the few tertiary bases with appreciable neuromuscular blocking activity and, being a tertiary base, is active orally, on the cat gastrocnemius Salama and Wright (1951) found the equipotent molar ratio relative to (+) tubocurarine chloride to be 85 The methiodide, however, is much less active (Unna and Greslin, 1944, Unna, Kniasuk, and Greslin, 1944), but activity is increased by reduction to the dihydro compound (V 60, Boekelheide, Weinstock, Grundon, Sauvage, and Agnello, 1953),

Salama and Wright obtained an equipotent molar ratio of 8 5 on the cat gastrocnemius and Bovet *et al* (1949) found the ratio to be 28 in the rabbit head-drop test

The absolute configuration of both β-erythroidine and dihydro-β-erythroidine has recently been established Wenzinger and Boekelheide (1963) have shown by chemical methods that the former has the 3R,5S configuration, as in V 59, and that the latter has the same arrangement, as in V 60, though
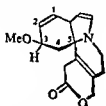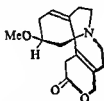
β-Erythroidine, V 59   Dihydro β Erythroidine, V 60

with the different position of the double bond, this will be described as 3S,5S Hanson (1963), using crystallographic methods, has worked out not only the relative configuration of the groups in the latter but also even their absolute configuration (which cannot usually be determined in this way) The results obtained by both methods were published simultaneously and are in agreement with each other

TABLE V 10

Neuromuscular Blocking Activity of Calabash curarine Alkaloids Approximate Equipotent Ratios Relative to (+)-Tubocurarine Chloride

	Cat gastrocnemius		Frog gastrocnemius (isolated)	Mouse head drop
	Threshold effects	Complete block		
C-alkaloid E	0 17	0 056	0 006	0 004–0 053
C-alkaloid G	0 17	0 14	0 01	0 008–0 067
Toxiferine I	0 25	0 060	0 06	0 12
C-alkaloid H	0 33	0 13	—	0 21
C-curarine I	0 42	0 20	0 03	0 40
C-alkaloid K (Dihydrotoxiferine, deoxytoxiferine I)	1 2	0 44	—	0 40
C-alkaloid A	3 3	1 4	2 4	0 93
Calebassine (Toxiferine II)	5 0	1 7	0 6	3 2

Waser (1953)

Because the molecular weights of the alkaloids are all approximately the same as those of (+)-tubocurarine chloride, the values in this table can be taken as equipotent molar ratios Paton and Perry (1951) obtained the following values for toxiferine I on the cat tibialis, 0 067, on the frog righting reflex, 0 013–0 004, on the mouse righting reflex, 0 08, in the rabbit head drop test, 0 05

Alkaloids of Calabash Curare

Recently considerable attention has been given to the chemistry and pharmacology of the alkaloids of calabash curare (reviews, Karrer, 1959, Battersby and Hodson, 1960, Waser, 1953, 1959) The most active of these are all *bis* quaternary ammonium salts Approximate equipotent ratios are shown in Table V 10 and, as the compounds all have molecular weights very similar to that of (+)-tubocurarine chloride, the values can be taken as equipotent molar ratios The structures of Toxiferine I (V 61), C-alkaloid H (V 62), and C-alkaloid K (dihydrotoxiferine I, V 63) have been worked out by Arnold *et al* (1961), that of Toxiferine II (C-Calebassine, V 64) by Hesse *et al* (1961), and those of two much less active compounds, Caracurine II (V 65) and C-alkaloid D (V 66) by Battersby *et al* (1961) These all contain two of the same fused 5 ring units joined together in different ways It is possible that

V 61–66 These all contain two of the units
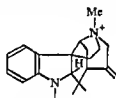
joined in the following ways
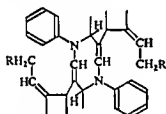
$R_1 = R_2$ OH V 61
$R_1 = H$ $R_2 = $ OH V 62
$R_1 = R_2 = H$, V 63
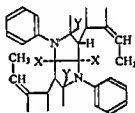
X = H Y = OH
V 64
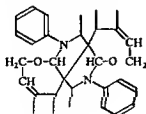
V 65
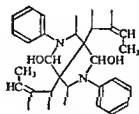
V 66

high activity is associated with a relatively flexible bridge (compare the structures of Toxiferine I, Toxiferine II and C alkaloid D) C Alkaloid D is only feebly active and is particularly rigidly clamped by the 16—16 bond in this molecule the distance between the onium groups is only 8 6 Å (McPhail and Sim, 1961), whereas in Toxiferine I it has been calculated to be about 14 Å as in (+) tubocurarine (Battersby and Hodson, 1960) It is interesting that

in spite of the complexity of these structures, the onium group is not 'buried' inside the molecule, but is relatively accessible (this point is more easily appreciated by an examination of models)

The high activity of C-alkaloid E is very striking it appears to be the most active neuromuscular blocking agent so far described, although its activity relative to Decamethonium or the dimethyl ether of $(+)$ tubocurarine will vary greatly from one species to another

Distribution of Receptors at the Neuromuscular Junction

From experiments with labelled Calabash Curarine, Waser (1959) concluded that 8×10^6 molecules of this compound were needed to block a single end-plate in the mouse diaphragm As stimulation of a single nerve-ending leads to the release of about 5×10^5 molecules of acetylcholine, he suggested that there are not more than about 10^7 receptors per end plate and that for a maximal response less than one tenth of these are occupied (cf page 8) The end plate appears to have, very roughly, an area of 2,000 μ^2 (Cole, 1957, Miledi, 1960), this works out at 1 receptor per 2×10^4 Å^2 Although this estimate is obviously only a drastic approximation it indicates an order of magnitude which is reasonably consistent with other results Cohen and Warringa (1953) estimated that there were 520 cholinesterase active centres per ox red cell (page 279) Assuming the surface area of the latter to be 120 μ^2, this works out at 1 receptor per 2×10^7 Å^2 Unless there is evidence that the receptors are arranged together in groups on the surface of either of these structures – and the results of Miledi (1960) suggest that this is unlikely – it must be supposed that the distance between receptors is far too big for a single drug molecule to interact with more than one at once

The Structure of Receptors at the Neuromuscular Junction

Most antagonists at the neuromuscular junction are relatively large molecules, like the alkaloids of calabash and tube curare whereas most agonists are either small, like acetylcholine, or slender, like Decamethonium As will be seen later, this is true also of other sites where acetylcholine is active To account for the difference between the activity of, say, acetylcholine and tetramethylammonium, it seems reasonable to suppose that the receptor consists of more than one group and may, in fact, where agonists are known which are stereospecific, consist of at least three groups These receptor groups which comprise the receptor proper must lie within at the most 10 Å of each other (the length of an extended molecule of acetylcholine) The material surrounding the receptor, however, must also be considered, it cannot be thought of simply as a featureless surface of uniform electron density If, for instance, it is part of a protein structure there will be peptide bonds and possibly amino, hydroxyl or sulphydryl groups close enough for these to interact with groups on the larger antagonist molecules These anchoring sites (Gill and Ing 1958) could account for the high affinity of antagonists

It should, in theory, be possible to infer the existence of anchoring sites from the variation of neuromuscular blocking activity with chemical structure,

but there are two difficulties, the flexibility of many of the molecules and the possibility that the combining sites are distributed in all directions about the receptor proper In a series of compounds where there is a sharp maximum in activity at a particular chain length, it seems reasonable to suppose that this maximum coincides with ability to combine with the receptor and an anchoring site simultaneously

The number of neuromuscular blocking agents which have a second onium group about 13–15 Å away from the first suggests the existence of a negative group on the receptor surface in this position In other series of neuromuscular blocking agents, however, the position of the maximum is different (e g in the polymethylene biscarbamic esters of choline and in polymethylene bis-triethylammonium salts) and the activity increases much more gradually with chain length The contribution to adsorption of van der Waals' forces, however, cannot be ignored, and in these large molecules it is conceivable that each extra methylene group in the chain, giving rise to an additional van der Waals bond, contributes a particular increment to the affinity (cf page 211) It might, therefore, be expected that activity would increase geometrically with chain length, and this appears to be more or less true in these series of biscarbamic esters and *bis* triethylammonium salts

As there do not appear to be any agonists which show stereospecificity at the neuromuscular junction, it is difficult to decide whether the receptor unit consists of two groups or three, but there is little doubt, from the stereospecificity of the antagonists, that these are attached at more than two points

Differences Between the Neuromuscular Junction and Ganglia

It is in the arrangement of these anchoring sites that neuromuscular junctions and ganglia differ most distinctly Although the receptor groups at these different sites are not arranged identically, acetylcholine is active at both Antagonists, on the other hand, are much more selective, in cats, Decamethonium does not affect ganglia in doses 100 times those which block the neuromuscular junction, and Hexamethonium does not affect the neuromuscular junction in doses 100 times those which block ganglia (Paton and Zaimis, 1949) Likewise ten times as much (+) tubocurarine chloride was needed to block the cat's superior cervical ganglion as blocks the end plate of the cat's tibialis, for *Gaskalous E* and *G* the ratio is *B* and for *Toxiferine I* it is 80 (Waser, 1953) On the other hand, some of the less active alkaloids are less specific, as are the polymethylene *bis* trimethylammonium salts which may not be involved in attachment at an anchoring site

Possible Connexion Between Ability to Cause Contracture and Ability to Block by Desensitization

The relationships between chemical structure and ability to cause contracture of the frog rectus have already been discussed (page 117), and it seems likely that there must be some connexion between this and ability to block by desensitization Katz and Thesleff (1957) have suggested that perhaps the

receptor can exist in two interconvertible forms, A and B, and that drugs usually have a greater affinity for B than for A, that only the combination with form A leads to a response and that the rate of interconversion of either the complex with A to the complex with B, or of B alone back to A, is slow compared with the achievement of equilibrium by the drug with either form of receptor This can be written

$$Drug + A \rightleftharpoons Drug\ A\ complex$$
$$\uparrow (slow) \qquad \downarrow (slow)$$
$$Drug + B \rightleftharpoons Drug\text{-}B\ complex$$
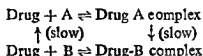
This hypothesis receives support from studies of the rate of onset of block and the rate of recovery afterwards If it is correct, the possibility of desensitization will depend upon the ratio of the affinity constants, K_B K_A If the compound has a much higher affinity for B than A it will tend to desensitize, whereas if the ratio is not so high it will be more difficult to produce desensitization An action like acetylcholine will normally depend upon a combination with type A receptors which leads to depolarization and a response If, however, the concentration is allowed to build up there may be a block in transmission by combination with type B receptors

The action of Decamethonium certainly appears to be a two stage process Holmes, Jenden, and Taylor (1951) and Jenden, Kamijo, and Taylor (1951, review by Taylor, 1951) concluded that the first stage, which in the rat diaphragm was the rate controlling step, was a diffusion, a truly reversible dissociation between drug and the receptors This was not a diffusion to the neuromuscular junction, such as was investigated by Ing and Wright (1931) (page 97), but a process occurring at some point or surface within the neuromuscular junction itself This first phase corresponded with the depolarization of the end plate It was unaffected by an anticholinesterase or by adding K^+ ions, and was followed *after* some *considerable time* by a block which resembled that produced by (+)-tubocurarine

It might be supposed that this first phase corresponds with depolarization block due to an action at type A receptors and the latter to an action at type B The picture is by no means clear, however, because the desensitization, i e the reduction in the end plate potential produced by Decamethonium, succinylcholine or even acetylcholine adds on to that produced by a competitive blocking agent such as Gallamine triethiodide, contrariwise in certain circumstances application of acetylcholine deepens the block produced by Gallamine triethiodide (Fig V 10, Thesleff, 1958) This is not what would be expected if the compounds were acting by depolarization Certainly the contracture of slow fibres produced by Decamethonium or succinylcholine is antagonized by substances like (+) tubocurarine, and Dallemagne and Philippot (1952) have shown that (+) tubocurarine can antagonize the effects of Decamethonium on the cat tibialis and soleus muscles

It seems, then, that it is only in the later stages that the two different types of blocking agent act additively, and how long it takes before these 'later stages' are reached appears to depend very much upon the species and muscle

concerned and on the rate of stimulation (Axelsson and Thesleff, 1958, Thesleff, 1960) It is very easy, for instance, to desensitize the end-plates of the cat's tenuissimus muscle It has been suggested that perhaps Decamethonium, being a slender ion, can penetrate to some site which is inaccessible to the much larger (+)-tubocurarine ion (Taylor, 1951) and perhaps this accounts for the second stage of block It is conceivable that if Decamethonium were producing a block by fixing the receptors in the inactive B form, the block would be increased by (+)-tubocurarine combining with the limited number of type A receptors The disadvantage of this type of picture, and of that suggested by Waser (1959) in which Decamethonium is supposed to act by bridging the mouth of a 'pore' but failing to plug it properly, is that involves a mechanical response by the receptor This may well be the way in which these drugs act, but unfortunately it involves speculation about processes for which there are as yet no satisfactory models in physical chemistry
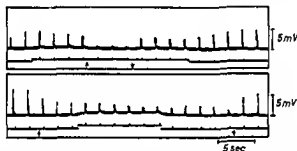
FIG V 10   *Addition of desensitizations produced by Gallamine triethiodide and acetylcholine the spikes are produced by the brief electrophoretic application of acetylcholine to the end plate of the cat's tenuissimus muscle The upper picture shows the effects of Gallamine triethiodide (added at the arrow and washed out at the second arrow) on the desensitization produced by acetylcholine (whose presence is indicated by the upward movement of the lower line) The lower picture shows the effects of acetylcholine on those of Gallamine triethiodide (the presence of the Gallamine triethiodide again being indicated by the arrows and of the acetylcholine by the movement of the lower line) (Thesleff, 1958 reproduced by permission)*

'Mixed Block'

The ability of a compound to act apparently by more than one mechanism has already been mentioned (page 114) With all the compounds of this type studied so far, the change is from a spastic paralysis to a flaccid, i e from a contracture or acetylcholine like effect to an apparently (+) tubocurarine like effect which may actually be a desensitization This is not merely dependent on the drug acting at different rates on different types of muscle by either one type of action or the other because it can be observed even on a single type of muscle Some of the higher homologues of Decamethonium initially cause contracture of the chick biventer, but the contracture passes off even in the presence of the drug (Decamethonium itself will do this if left long enough) This, then, can be explained by supposing that the subsequent

block is a desensitization, depending on the drug gradually penetrating to some deep-seated receptors and the ability to cause 'mixed block' will depend upon the relative affinity of the drug for the two types of receptor and ability to diffuse to the second type

For a discussion of events occurring at the receptor in terms of 'rate' theory (page 16) see Paton and Waud (1962), and for suggestions about the nature of the receptor itself see Cavallito (1962)

Antagonists of Neuromuscular Blocking Agents

When an overdose of (+)-tubocurarine has been given, an anticholinesterase (Chapter VIII) can be given as an 'antidote' It may be questioned whether this treatment is superior to artificial respiration with a machine (especially as the anticholinesterase will produce all the autonomic symptoms of cholinergic stimulation), but this may not be available There is no satisfactory antidote
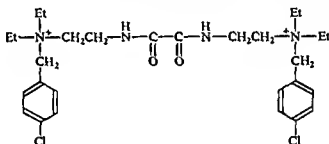
Ambenonium, V 67

for Decamethonium or succinylcholine, although some compounds have been described as antagonizing their effects Paton and Zaimis (1949) reported that Pentamethonium would antagonize the block produced by Decamethonium, but its ganglion blocking properties prevented its use for this purpose clinically De Beer *et al* (1951) and Ellis *et al* (1953) have also described compounds which reversed its action It seems that this reversal, which occurs during the early stages of the block by Decamethonium, can be ascribed to the (+)-tubocurarine *like* properties of the compounds (cf page 135) If the block produced by Decamethonium or succinylcholine persists for a number of days, it has been claimed that it may be reversed by an anticholinesterase (Foldes, 1959), but this treatment may be very hazardous if used to try to reverse the effects of excess succinylcholine too soon Anticholinesterases, such as eserine or Neostigmine, have appreciable activity on the butyryl-cholinesterases of serum as well as on the 'true' acetylcholinesterase of the neuromuscular junction and hence may block the destruction of succinylcholine by the serum enzyme Some substances (e g *Ambenonium, Mytelase, Win* 8077, V 67, Karczmar, 1957) appear to be able to antagonize both types of drug These have anticholinesterase-activity and blocking activity like that of (+) tubocurarine As anticholinesterases they reverse the effects of (+) tubocurarine and in higher concentrations, as (+)-tubocurarine-like blocking agents, they reverse the action of Decamethonium in its

VI

Actions at Cholinergic Synapses:
II. Autonomic Ganglia

Transmission in ganglia – Antagonism of the action of acetylcholine in ganglia –
Desensitization – Uses of ganglion blocking agents – Testing of drugs on ganglia,
preparations – Agonist activity – Blocking activity

AGONISTS Activity of simple onium salts – Effects of altering the onium group in
acetylcholine – Effects of altering the acyl group – Effects of altering the choline
part of acetylcholine – Nicotine and related compounds – Dimethylphenyl
piperazinium – Tropine derivatives, *N-417* – Relationships between structure and
ganglion stimulant activity

ANTAGONISTS (+)-tubocurarine and tetraethylammonium – Simple onium
salts – *Trimetaphan* – Tropine derivatives – Other ganglion blocking onium salts
used in the treatment of gastric and duodenal ulcers – *Bis-onium* salts *Pentame-
thonium* and Hexamethonium – Compounds related to Hexamethonium – Effects of
altering the polymethylene chain – *Azamethonium* – Asymmetrical *bis-onium* salts –
Ganglion blocking compounds which are secondary or tertiary bases

Specificity of drugs for particular ganglia – Relationships between structure and
ganglion blocking activity – Actions at ganglia other than at receptors on the
postganglionic neurone – Conclusion

Transmission in Ganglia

Transmission in ganglia is, in many respects, very similar to transmission at
the neuromuscular junction The transmitter, acetylcholine, is released from
the endings of the presynaptic fibres and acts on postganglionic neurones
giving rise to an action potential in the postganglionic fibres It is possible,
with certain ganglia, to place electrodes on the pre- and/or post-ganglionic
fibres and on the ganglion itself and to observe the depolarization of the
ganglion produced by stimulation of the preganglionic fibre It is even possible,
in some ganglia (Nishi and Koketsu, 1960), to place a micro-electrode inside
the postganglionic neurone and to record electrical events in a manner
similar to that at the neuromuscular junction Blackman, Ginsborg,
and Ray (1963) have shown that, using this type of intracellular recording,
small spontaneous depolarizations occur quite frequently in the sympathetic
ganglia of the frog These miniature synaptic potentials can be compared to
the miniature end-plate potentials occurring at the neuromuscular junction
(page 88) Blackman, Ginsborg and Ray (1963) have shown that, using this type of intracellular recording,
and it is suggested that the synaptic potential itself, that is the
depolarization produced by stimulating the preganglionic nerve, is com-
pounded of a number of these miniature potentials, just as the end plate
potential at the neuromuscular junction is compounded of a number of
miniature end-plate potentials The release of acetylcholine would seem
therefore, to be quantal in the ganglion as well as at the neuromuscular
junction

The distribution of acetylcholinesterase, however, in the ganglion does not follow the same pattern as in the neuromuscular junction (Koelle and Koelle, 1959) and enzymic destruction of the transmitter seems to be much less important in the ganglion than at the neuromuscular junction. At the latter the enzyme is located on the membrane of the muscle end plate, but in the ganglion there are appreciable amounts on the surface of the presynaptic fibres. If the postganglionic fibre is adrenergic, the acetylcholinesterase located on the preganglionic fibre is about the only source of enzyme in the ganglion cell. If the postganglionic fibre is cholinergic, however, there is also acetylcholinesterase in the cytoplasm of the postganglionic fibre and a certain amount (a lot in the ciliary ganglion, but less in the stellate ganglion) on the surface of the postganglionic neurone.

Antagonism of the Action of Acetylcholine in Ganglia

The action of acetylcholine, producing a synaptic potential and an action potential in the postganglionic fibre, is antagonized by a number of compounds, including (+) tubocurarine. Although the dose of (+) tubocurarine chloride which blocks ganglia is about ten times that which blocks the neuromuscular junction, its action in the ganglion is always assumed to be by competition with acetylcholine. Although this may well be correct, there is no direct evidence for this, there have so far, been no experiments with a ganglion comparable with those of Jenkinson (1960) with the frog sartorius.

Desensitization

The similarity between the ganglion and the neuromuscular junction extends to the action of large amounts of acetylcholine. Paton and Perry (1953) and Perry and Talesnik (1953) showed that these produced depolarization of the ganglion and a reduction in the synaptic potential (Fig VI 1) There was some degree of correlation between the degree of depolarization and the reduction of the synaptic potential, especially during the early phases of the block, but the block may subsequently persist for some time after the repolarization of the ganglion and can better be ascribed to desensitization than to depolarization. This type of action is also observed with nicotine, whose ability to stimulate and subsequently block autonomic ganglia was noted by Langley and Dickinson as long ago as 1889

Compounds acting on the receptors in ganglia can accordingly be divided into two groups substances which act like acetylcholine, initially stimulating the ganglion and subsequently blocking by depolarization and desensitization, and substances which antagonize the actions of acetylcholine and cause a block in transmission. The difference between the two types is more easily seen than at the neuromuscular junction, because the transition from stimulation to desensitization does not occur so readily in the ganglion as it does in the neuromuscular junction. For a review of the actions of drugs at ganglia see Paton (1954)

Uses of Ganglion-blocking Agents

One of the main effects of ganglion blocking agents *is to cause a fall in* blood pressure. Although both sympathetic and parasympathetic ganglia

are blocked to about the same extent, the level of the blood-pressure appears
to be determined primarily by the sympathetic tone, the parasympathetic
tone being much less important. The effects of a ganglion-blocking agent is,
therefore, to lower the blood-pressure, particularly where this is set at a
high level because the sympathetic tone is high One method of dealing with
a raised sympathetic tone is to cut the sympathetic nerve supply, but this
surgical treatment is not always successful (the high blood-pressure could
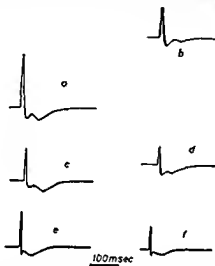
FIG VI 1 *Effects of drugs on electrical events in ganglia*
    *The records are of electrical events in the ciliary ganglion of the cat, with the
    electrodes placed one on the cut end of the nerve and the other
    round the body of the ganglion The traces a, c, and e were obtained before the
    addition of a drug and b, d, and f after the intra arterial injections of acetylcholine,
    nicotine, and hexamethonium, respectively With acetylcholine the trace moves
    upwards, indicating depolarization of the ganglion, and there is a slight reduction in
    the synaptic action potential with nicotine, likewise, there is both depolarization
    and a reduction in the size of the synaptic action potential, but with hexamethonium
    there is a reduction in the size of the action potential without any depolarization
    (Perry and Talesnik, 1953)*

have other causes besides a raised sympathetic tone), and at one time the
short-lasting ganglion blocking effects of tetraethylammonium were made use
of to assess in the likely results of sympathectomy This use of
tetraethylammonium was not always very satisfactory (Lyons, Hoobler,
Neligh, Moe, and Peet, 1948) Long-lasting ganglion blocking agents, how-
ever, have been found to be of considerable value in the treatment of condi-
tions where the blood-pressure is high (Organe, Paton, and Zaimis, 1949)

    When the sympathetic ganglia are blocked the blood tends to 'pool' under
the influence of gravity, and one disadvantage of this treatment is that when
a patient stands up suddenly he may faint because of the inadequate blood-
supply to the brain This effect can, however, be made use of by the anaes-
thetist to reduce bleeding in surgery A ganglion blocking agent is injected
and the patient tilted so that the part to be operated on (e g the head) is

raised and the blood collects in the lower limbs Drugs used for this procedure must produce only short-lasting effects because it is undesirable either to reduce the blood supply to any part of the body for too long or to allow the blood-pressure generally to fall during anaesthesia Therapeutically they must be considered as a separate class from drugs used in the treatment of high blood-pressure

Ganglion-blocking activity may also be useful in compounds designed for the treatment of gastric and duodenal ulcers The aim is to reduce the motility of the gut and stomach and the secretion of acid gastric juice The compounds normally employed for this purpose act at the postganglionic parasympathetic receptors on the smooth muscle (see Chapter VII), but ability to block ganglia may also be advantageous, particularly for reducing the peristaltic movement of the gut, and several substances have been developed which combine blocking activity at both the postganglionic parasympathetic receptors and at ganglia

Testing of Drugs on Ganglia

Preparations

It is more difficult to test the action of drugs on ganglia than at the neuromuscular junction because ganglia are usually much more difficult to get at and to keep alive This is particularly true of parasympathetic ganglia which, with a few exceptions, are located on the muscle or organ they innervate It is not possible to separate the ganglion cell from the postganglionic fibres and postganglionic synapses with the muscle or organ, and consequently the same responses could be produced by drugs acting on the postganglionic fibre and at the postganglionic receptors on the muscle or organ as well as at the ganglion itself The sympathetic ganglia, on the other hand, are usually some distance from the muscle or organ they innervate and yield less ambiguous information about the action of drugs, but even these demand much more skill in their preparation than do most neuromuscular preparations

The most commonly used ganglion preparation is the sympathetic superior cervical ganglion of the cat (Kibjakow, 1933) This is situated in the neck and innervates the nictitating membrane (the third eyelid) The blood-supply is *reasonably accessible and consequently the ganglion can be perfused if* necessary, otherwise drugs can be injected intra-arterially close to the ganglion Electrical stimulation of the preganglionic nerve produces a synaptic potential and a depolarization of the ganglion, which can be detected with external electrodes (Paton and Perry, 1953), and a contracture of the nictitating membrane, which can be recorded using a thread led over a pulley and attached to a lever The extent of the contracture will depend (among other things) on the number of postganglionic neurones in the ganglion which are stimulated by the release of transmitter from the preganglionic nerve-endings

The most direct information about the action of drugs on parasympathetic ganglia is obtained from the ciliary ganglion preparation described by Whittendge (1937) This is unusual among parasympathetic ganglia in that it is some distance from the ciliary body and iris (in the eye) which it

innervates The effects of drugs can be studied on the electrical events in the ganglion, using external electrodes, or on the size of the pupil, which constricts in response to parasympathetic stimulation (Perry and Talesnik, 1953)

The stellate ganglion, whose postganglionic fibres are mostly adrenergic and run to the heart, the inferior mesenteric ganglion, from which adrenergic fibres innervate the bladder and rectum, and the sphenopalatine ganglion, whose postganglionic fibres are mostly cholinergic and innervate the lachrymal gland, are all suitable for testing ganglion blocking agents, but have not been much used
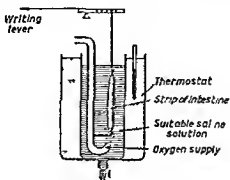
FIG VI 2   *Isolated intestine in the original preparation described by Magnus (1904) the intestine lay horizontally in a dish and the thread ran over a pulley to the lever*

The activity of drugs on parasympathetic ganglia has mostly been studied on preparations, such as the isolated guinea pig ileum or the perfused heart, in which the ganglia have not been separated from the postganglionic receptors on the muscle or organ The ileum can be mounted in two ways In one (Magnus, 1904), only the contractions of the longitudinal muscle are recorded (Fig VI 2), but in the other (Trendelenburg, 1917), the ileum is mounted so as to record the peristaltic reflex Magnus actually used mostly cat intestine, but guinea pig ileum is much more suitable as there is far less spontaneous activity This preparation is also suitable for 'superfusing' in the manner described by Gaddum (1953) In the Trendelenburg preparation the proximal end (nearest the stomach) of the piece of ileum is tied off, like a sausage, and attached to a lever The anal end is fitted over a glass tube attached by rubber tubing to a reservoir containing a suitable salt solution, usually Tyrode's solution, and connected to a piston type volume recorder The outside of the ileum is also immersed in Tyrode's solution, but the supply to the inside is separate from that to the outside If the pressure inside the ileum is raised by raising the reservoir, this stimulus may set up the peristaltic reflex, giving rise to waves of peristalsis in which both the circular muscle and the longitudinal muscle of the ileum are involved The changes in volume, arising mostly from contraction of the circular muscle, are recorded by the piston recorder and changes in length by the movements of the lever An improved version of this apparatus, in which drugs can be

applied to the inside of the ileum, as well as to the outside, has been described by Bulbring, Crema, and Saxby (1958)

In the preparations so far described, the drug can be applied close to its site of action, this should be an advantage when fundamental information about the action of the drug is required Information about the possible clinical value of a drug, however, is more likely to be obtained from more complicated experiments using whole animals Drugs are often tested for their effects on the blood pressure of anaesthetized, or even conscious, animals, but changes in pressure could be brought about by actions at a variety of sites other than ganglia If the drug is, in fact, acting at ganglia, its effects on the blood-pressure will be brought about largely by actions on sympathetic ganglia and possibly by stimulating the adrenal medulla The extent to which the latter may be involved can be determined in experimental animals by observing the effects of tying off the blood supply to the adrenal glands

To assess the effects of drugs on parasympathetic ganglia in whole animals, the size of the pupil of the eye of the mouse has been employed (Edge, 1953)

Agonist Activity

The agonist activity of a drug at ganglia can be expressed in terms of the equipotent molar ratio relative to some other agonist Ideally this could be done by comparing the concentrations of the two drugs which produced comparable synaptic potentials or comparable degrees of depolarization of the ganglion Alternatively the size of the contracture of the nictitating membrane could be used, when drugs are injected into the arterial supply to the cat's superior cervical ganglion, or the change in the pupil size, when drugs are tested on the ciliary ganglion Although the drugs might produce a response by acting directly on the postganglionic receptors on the nictitating membrane or iris, there is little likelihood of this happening, because the drug is applied close to the ganglion and little escapes into the general circulation It is possible to use the other nictitating membrane or iris as a control to check this point

Agonist activity at ganglia will result in contraction of the guinea pig ileum, rise in blood pressure and constriction of the pupil All these, like the effects on the superior cervical and ciliary ganglia already mentioned, are graded responses and could be used for attempts to determine equipotent molar ratios relative to some standard but with these preparations it must be clearly established that the effects really are the consequence of actions at ganglia and not at other sites The effects on the blood pressure are often tested in the presence of atropine, which blocks the postganglionic parasympathetic receptors, and hence only the effects on the sympathetic system are seen

One difficulty in the way of obtaining equipotent molar ratios for stimulant activity on ganglia is the tendency for high doses to produce some degree of ganglion block This for instance, upsets the performance of a 4-point assay

Blocking Activity

Blocking activity at ganglia could be studied in the same way as blocking activity at the neuromuscular junction Theoretically it should be possible to determine the association constant for the antagonist and the receptors, for instance, by measuring the degree of depolarization produced by a particular amount of acetylcholine and finding the concentration of the antagonist in the presence of which double the amount of acetylcholine produced exactly the same effect (cf the experiments of Jenkinson, 1960, described on page 92) This type of experiment could also be performed using the gross pharmacological response, contracture of the nictitating membrane, instead of the electrical response It could also be done on the guinea-pig ileum, or on the blood-pressure, provided a ganglion-stimulant were used which had no effect on the postganglionic receptors Acetylcholine, for instance, would be unsuitable because it affects the latter in concentrations lower than those which affect the ganglion This determination of the antagonist constant for ganglion blocking drugs does not seem to have been attempted

Antagonist activity has mostly been expressed relative to that of another antagonist drug by comparing the concentrations producing comparable degrees of ganglion-block This may be assessed by measuring electrically the depolarization of the ganglia or observing the effect of the drug on reducing the size of the contracture of the nictitating membrane produced by preganglionic stimulation (Hunt and Renshaw, 1925, Chou and Eho, 1947, Paton and Zaimis, 1949) Similarly, if the isolated guinea pig ileum is treated with a 'specific' ganglion stimulant, contractions should be obtained, and the percentage reduction in the size of these brought about by an antagonist could be used as a measure of its effect (Fakstorp and Pedersen, 1954)

On the other preparations it is not necessary to add an agonist in order for the antagonist to produce an effect which can be seen In the whole mouse, the effect of a drug in blocking the parasympathetic ciliary ganglion will be to upset the balance between the sympathetic and parasympathetic stimulation of the iris The circular muscle will offer less opposition to the sympathetically innervated radial muscle and the pupil will become dilated Likewise in the cat, the action of drugs in blocking the sympathetic ganglia will lead to a fall in blood-pressure Either of these effects, dilation of the pupil or fall in blood-pressure, could be used for comparing the activity of ganglion-blocking drugs, but it should be recognized that, in addition to the possibility that the result is produced by an action at sites other than ganglia, the magnitude of the effect depends upon the relative physiological importance of the sympathetic and parasympathetic systems in the animal, as well as on the degree of block in the ganglia In an animal with a high sympathetic tone the effects of a ganglion-blocking agent on the blood pressure will be greater than in others with lower sympathetic tone It might be expected, therefore, that the responses to a particular dose of a drug on this type of preparation would vary considerably from one animal to another

Another method of avoiding the addition of an agonist to stimulate the

ganglion is to stimulate sensory pathways leading to a ganglion involved in a reflex arc The peristaltic reflex is particularly convenient, being a local reflex which does not proceed through the spinal cord An increase in pressure inside the gut stimulates sensory receptors in the wall of the gut, sensory impulses are carried to the ganglia in the plexuses of the gut, and from these impulses travelling motor postganglionic fibres stimulate the appropriate muscle fibres Drugs may block the reflex by blocking conduction of impulses along nerve-fibres (page 53), by blocking transmission at the ganglionic synapse or by blocking transmission at the postganglionic receptors on the muscle The preparation has been used for estimating ganglion blocking activity by comparing the concentrations which abolish the reflex, in particular the response of the circular muscle (Feldberg and Lin, 1949, Paton and Zaimis, 1949, Kosterlitz and Robinson, 1957), but it is difficult to obtain very accurate results with it The response to a particular rise in pressure is rather variable and it is difficult to wash the preparation without causing pressure changes which disturb it

Ganglion blocking drugs can also be tested for their ability to block the carotid occlusion reflex, i e the reflex rise in blood pressure produced in response to occlusion of the carotid artery, although this technique has actually been used more for testing compounds which interfere with the release of the sympathetic transmitter from the postganglionic nerve-endings (Maxwell, Ross, and Plummer, 1958, see page 341)

AGONISTS

Activity of Simple Onium Salts

Burn and Dale (1915) noted tetramethylammonium produced usually a fall, but occasionally a rise, in the blood pressure of the anaesthetized cat In the presence of atropine, however, it always caused a rise, which they concluded to be due to stimulation of sympathetic ganglia Tetramethylammonium on the other hand, did not cause a rise in pressure and in fact, antagonized the effects of tetramethylammonium Relative to acetylcholine, tetramethylammonium is very much more active at ganglia than at the neuromuscular junction Burn and Dale did not make a quantitative comparison, although they comment that tetramethylammonium was comparable with nicotine in activity (page 153) It is certainly more active than acetylcholine

Hunt and Renshaw (1925) also using the atropinized cat, found that the stimulant effects of tetramethylammonium were greater than those of tri methylsulphonium, which in turn were greater than those of tetramethyl phosphonium tetramethylarsonium and tetramethylstibonium appeared to be inactive The effects of some alkyltrimethylammonium salts were studied qualitatively by Hunt and Renshaw (1925) and Renshaw (1926) and more completely and quantitatively by Alles and Knoeffel (1939, quoted by Bovet and Bovet-Nitti, 1948) Activity appears to be maximal in the *n* pentyl and *n* hexyl compounds, which are more potent than tetramethylammonium (Table VI 1) Willey (1955) records an equipotent molar ratio for *n* pentyl trimethylammonium relative to acetylcholine of 0 12, and relative to nicotine

of 0 93, when compared for ability to raise the blood pressure of the spinal cat in the presence of atropine

Phenyltrimethylammonium, benzyltrimethylammonium and β-phenyl ethylammonium were shown to cause a rise in the blood pressure of the anaesthetized cat and dog (Alles, 1933) β Phenylethyltrimethylammonium was particularly active, being more powerful than tetramethylammonium and possibly more active than nicotine

Other onium salts are discussed in the section dealing with compounds related to nicotine

TABLE VI 1

Ganglion Stimulant Activity of Alkyltrimethylammonium Salts

	Approximate equipotent molar ratio relative to tetraethyl ammonium estimated by ability to raise the blood pressure of the anaesthetized dog
Me-$\overset{+}{N}$Me₃	1
Et	4
n Pr	20
n Bu	1
n-Pent	0 3
n Hex	0 2
n Hept	1

Alles and Knoeffel (1939) quoted by Bovet and Bovet Nitti (1948)

Effects of Altering the Onium Group in Acetylcholine

Analogues of acetylcholine in which the onium group has been altered have not been tested for ganglion stimulant activity as extensively as they have for acetylcholine like activity on the frog rectus It might be expected that the results on the two types of preparation would be similar since they are both sites of the nicotine like actions of acetylcholine and that quite small changes in the onium group would decrease ability to act like acetylcholine Holton and Ing (1949) found the equipotent molar ratio for acetoxyethyl ethyldimethylammonium (VI 1) relative to acetylcholine on the blood pressure

$$CH_3\ C\overset{\displaystyle O}{\underset{\displaystyle O\ CH_2CH_2}{\diagup}}\ \overset{\displaystyle Me}{\underset{\displaystyle Et}{\overset{+}{N}-Me}}$$
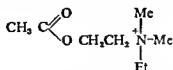
VI 1

of the anaesthetized cat, treated with atropine was 5 Further replacement of methyl by ethyl in the onium group gave compounds which were inactive Hunt and Renshaw (1925) reported that the phosphonium analogue of acetylcholine was inactive on this preparation

Effects of Altering the Acyl Group

Altering the acyl group in acetylcholine affects ability to cause a rise in blood pressure in the presence of atropine in much the same way as it affects ability to cause contracture of the frog rectus The relative activities of a number of esters of choline are shown in Table VI 2 Many aliphatic esters

TABLE VI 2

Ganglion-stimulant Activity of Esters of Choline Equipotent Molar Ratios Relative to Acetylcholine Determined by Comparing Doses Producing Comparable Increases in Blood pressure in Cats Treated with Atropine

ROCH₂CH₂NMe₃	H W	C and G	W	H and W	E and G	S and H*
R =						
H₂NCO (Carbachol)	0 31	—	—	—	—	—
CH₃CO (acetylcholine)	1 0	1 0	1 0	1 0	1 0	1 0
CH₃CH₂CO	0 65	0 5	0 8	—	—	0 25
CH₃CH₂CH₂CO	0 47	0 2	0 5	—	—	0 10
Me₂CHCO	0 26	—	—	—	—	0 17
CH₃CH₂CH₂CH₂CO	0 44	—	—	—	—	0 30
Me₂CHCH₂CO	—	—	—	—	—	0 15
Me₃CCO	0 10	—	—	—	—	—
Me₂C=CHCO	—	—	—	ca 0 1	—	0 050
MeHC=CHCO	—	—	—	⩾0 1	—	0 12
EtHC=CHCO	—	—	—	⩾0 1	—	0 19
H₂C=CMeCO	—	—	—	—	—	0 042
MeHC=CMeCO (tiglylcholine)	—	—	—	—	—	0 029
[structure]–CO	—	—	—	—	1 2	—
–CH₂CO	—	—	—	—	2 0	—
–CH₂CH₂CO	—	—	—	—	0 12	—
–CH–CHCO (murexine)	—	—	—	ca 0 5	—	—
–CH₂CH₂CO	—	—	—	—	1 3	—
[structure] –CH–CHCO	—	—	—	—	0 17	—
[structure] –CH–CHCO	—	—	—	—	0 17	—
–CH–CH₂CO	—	—	—	—	0 14	—
PhCO	0 88	—	2 3	—	—	—
PhCH₂CO	0 16	—	—	—	—	—
PhCH₂CH₂CO	—	—	—	—	—	0 12
PhCH=CHCO	—	—	—	—	—	0 053
PhCH=CMeCO	—	—	—	—	—	0 042
PhC=CCO	—	—	—	—	—	0 083

H W = Hey (1952) Willey (1955) C and G = Chang and Gaddum (1933) W = Wurzel (1959) H and W = Holmstedt and Whittaker (1958) E and G Erspamer and Glasser (1958) S and H = Sekul and Holland (1961) the asterisk is to indicate that these workers used dogs not cats

TABLE VI.2—*Continued*

Activity of Benzoyl Esters of Choline Equipotent Molar Ratios Relative to Benzoylcholine

Substituent	Blood pressure rise in the presence of atropine	Stimulation of superior cervical ganglion in the presence of eserine	S
m-NO$_2$	10	>10	+0 71
m-Cl	3 3	33	+0 37
m F	—	4 6	+0 34
p-Cl	2 2	2 5	+0 23
p-F	1 7	1 5	+0 06
m-Me	2 5	0 88	−0 07
p-Me	1 7	2 9	−0 17
p MeO	0 46	0 55	−0 27

Ormerod (1956) as the equipotent molar ratio for benzoylcholine relative to acetylcholine is close to 1 (0 9, Hey, 1952, 2 3, Wurzel, 1959), the figures in this table must be close to the values relative to acetylcholine

S is the Hammett constant, $\log\left(\dfrac{K}{K_s}\right)$, where K is the dissociation constant for the substituted benzoic acid and K_s that for benzoic acid itself

of choline are more active than acetylcholine itself and, in contrast to the situation with the frog rectus, even aromatic esters of choline show considerable activity The results are complicated by the different susceptibilities of the compounds to hydrolysis by cholinesterase, particularly, in the experiments on blood-pressure, to the butyrylcholinesterase of plasma More specific information about ganglion stimulant activity is obtained from experiments, such as those of Ormerod (1956), in which the drug is applied directly to the perfused ganglion in the presence of eserine Even more than in the discussion of acetylcholine like activity at the neuromuscular junction, the choice of acetylcholine as a standard for comparison can be criticized because of its susceptibility to cholinesterase It is the obvious choice, however, and with the development of preparations in which the drugs are applied directly to the ganglion, it should be possible to obtain results which are consistent

The most active compounds appear to be *n* butyrylcholine, trimethylacetylcholine, dimethylacrylylcholine, tiglylcholine, dihydromurexine, pyridazine-3-acryloyl- and -3 propionyl choline, cinnamoylcholine, and α-methylcinnamoylcholine Many of these, e g murexine (Keyl and Whittaker, 1958) and dimethylacrylyloylcholine (Holmstedt and Whittaker, 1958), are stable to cholinesterases, but *n* butyrylcholine, although only slightly affected by acetylcholinesterase, is hydrolysed twice as rapidly as acetylcholine by the butyrylcholinesterases of plasma (Adams and Whittaker, 1949) The high activity of this compound in raising the blood pressure suggests that the

results in Table VI 2 with this test really do give some idea of the activity of the compounds at the receptors in the ganglia

Although these compounds might be expected, like acetylcholine, to block transmission in ganglia in doses higher than those which cause stimulation, no results appear to be available

Effects of Altering the Choline Part of Acetylcholine

(±) Acetyl α methylcholine (VI 2) was found by Simonart (1932) to be as active as acetylcholine in raising the blood pressure of the anaesthetized cat in the presence of atropine. (±) Acetyl β methylcholine (VI 3), on the other hand, failed to produce any rise in pressure, this finding has been confirmed by Wurzel (1959)

$$CH_3\text{-}CO\text{-}O\text{-}CHR\text{-}CHR'\text{-}\overset{+}{N}Me_3$$
$$R = H \quad R' = Me, \text{ VI 2,}$$
$$R = Me \quad R' = H, \text{ VI 3}$$
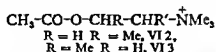
Esters of thiocholine are more active than their oxygen analogues Relative to acetylcholine, the equipotent molar ratios for the effects on the cat's blood pressure in the presence of atropine are 0 20 for acetylthiocholine, 0 22 for the propionyl ester, 0 12 for the butyryl ester, and 0 40 for the benzoyl ester (Wurzel, 1959) It is odd that, according to Renshaw, Dreisbach, Ziff, and Green (1938), acetyl β methylthiocholine is more than twice as active as acetylthiocholine in raising the blood pressure of the anaesthetized cat The α methyl compound does not appear to have been studied

Altering the length of the choline part of the molecule appears to reduce activity, both acetyl-γ-homocholine (VI 4, Hunt and Taveau, 1911) and acetylnorcholine (VI 5, Hunt and Renshaw, 1925) appear to be less active than acetylcholine

$$CH_3\text{-}C\overset{\displaystyle O}{\underset{\displaystyle O\text{-}(CH_2)_n\text{-}\overset{+}{N}Me_3}{\big\langle}}$$
$$n = 3, \text{ VI 4,}$$
$$n = 1, \text{ VI 5}$$
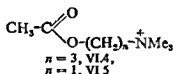
Aliphatic ethers of choline are relatively inactive in stimulating ganglia (Dale, 1914, Simonart, 1932, 1934) Willey (1955), for instance, recorded equipotent molar ratios relative to acetylcholine, on the blood pressure of the spinal cat in the presence of atropine, of 1 8 for the ethyl ether of choline and 0 53 for the *n* butyl ether Aromatic ethers, on the other hand, are extremely active Hey (1952) recorded an equipotent molar ratio for the phenyl ether of choline of 0 022 relative to acetylcholine and, if the adrenal glands were tied off, this ratio became as low as 0 012 Hey (1949) had suggested that 'maximum nicotine like stimulant action would be found in ions of the type $ROCH_2CH_2NMe_3$, where the character of the R group is such that maximum mesomeric deviation towards structures of the type –

$$\bar{R} \ \overset{+}{O}CH_2CH_2NMe_3 - would\ be\ expected'\ In the phenyl ether of choline,$$
as opposed to the aliphatic ethers, there will be an appreciable partial positive

F

charge on the ether oxygen atom because of the electron-withdrawing nature of the benzene ring Hey tested a number of substituted phenyl ethers of choline to see if there were any correlation between the electron-withdrawing or electron-releasing nature of the substituent and the ganglion-stimulant activity determined on the blood-pressure of the anaesthetized cat in the presence of atropine Some of the compounds are quite remarkably active (Table VI 3)

TABLE VI 3

Ganglion stimulant Activity of Substituted Phenyl Ethers of Choline

$$R \underset{}{\underbrace{\bigcirc}} O-CH_2CH_2NMe_3$$
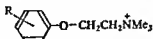
Equipotent Molar Ratios Relative to Acetylcholine Obtained by Comparing Doses Producing a 60 mm Rise in Blood pressure

R =	Adrenal glands intact	Adrenal glands ligated	S
3 5 Dibromo-	0·0065	0·0045	+ 0 8
m Bromo-	0 0060	0·0046	+ 0 39
m-Chloro-	0 010	0·0062	+ 0 37
H- (unsubstituted)	0 022	0 012	0
m-Methyl	0 17	0 089	− 0 04
p-Chloro-	0 21	0 12	+ 0 21
3 5 Dimethyl-	0 42	0 23	− 0 12
p-Methyl	5 5	2 6	− 0 14

The change in the ratio brought about by tying off the blood supply to the adrenal glands suggests that the contribution to the total rise in pressure from stimulation of the adrenals varies from drug to drug This might imply that the receptors in the gland are slightly different from those in sympathetic ganglia, but it could be explained by differences in the metabolism of the compounds If the activities are compared relative to choline phenyl ether, tying off the adrenals has little effect except to the ratio for acetylcholine itself S is the Hammett constant for the analogous benzoic acids (see Table VI.2)

Hey (1952)

The extremely high activity of the 3 5-dibromophenyl and m bromophenyl ethers of choline has been confirmed by Ambache and Robertson (1953) When tested on the perfused superior cervical ganglion of the cat, these substances caused stimulation and a contracture of the nictitating membrane but, in slightly higher doses, produced a block in transmission The results suggested that the compounds were, very roughly, equiactive and that the m bromophenyl ether of choline was between two and ten times as active as nicotine (see below)

In contrast to the aliphatic ethers, aliphatic ketones display considerable activity, Ing, Kordik, and Tudor Williams (1952) reported that 3- and 4-oxo-n-pentyltrimethylammonium (VI 6 and 7) only produced a rise in blood-pressure in the anaesthetized cat whereas 3-oxo-n-butyltrimethylammonium

(VI 8), the most active of these on the frog rectus (page 108), initially caused a fall in blood pressure The latter caused a rise in blood pressure in the presence of atropine, but was less active than the oxo *n* pentyl compounds Willey (1955) obtained an equipotent molar ratio of 0 041 for 4-oxo *n* pentyl trimethylammonium relative to acetylcholine on the blood pressure of the spinal cat in the presence of atropine

$$CH_3CH_2-\overset{O}{\overset{\|}{C}}-CH\cdot CH_2\cdot\overset{+}{N}Me_3 \qquad CH_3-\overset{O}{\overset{\|}{C}}-CH_2CH_2\cdot\overset{+}{N}Me_3$$
$$\text{VI 6} \qquad\qquad\qquad\qquad \text{VI 7}$$

$$CH_3-\overset{O}{\overset{\|}{C}}-CH_2CH_2\overset{+}{N}Me_3$$
$$\text{VI 8}$$
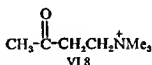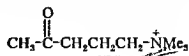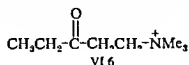
A number of compounds related to acetylcholine and containing hydroxyl groups and/or ethylenic or acetylenic bonds has been studied by Jacob *et al* (1952) The ganglion stimulant activity of some of these, 5 hydroxy and 4-hydroxy *n* pentyltrimethylammonium and 4-hydroxy *n* pent-2-ynyltrimethylammonium (VI 9, 10, and 11) is greater than that of acetylcholine
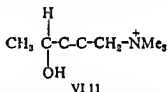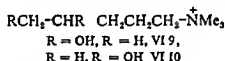
$$RCH_2\text{-}CHR \quad CH_2CH_2CH_2\cdot\overset{+}{N}Me_3 \qquad CH_3\ \overset{H}{\underset{OH}{\overset{|}{\underset{|}{C}}}}\text{-}C\text{-}C\text{-}CH_2\cdot\overset{+}{N}Me_3$$
$$R = OH, R = H, \text{VI 9},$$
$$R = H, R = OH \text{ VI 10} \qquad\qquad\qquad \text{VI 11}$$

The equipotent molar ratios relative to acetylcholine on the blood pressure of the anaesthetized dog in the presence of atropine lie between 0 2 and 1 0 It must be remembered, however, that *n* pentyltrimethylammonium itself is much more active than acetylcholine (equipotent molar ratio 0 12 page 147) so the results cannot be taken to imply that a 4 hydroxyl group or 2 acetylenic link confer activity on the molecule

Nicotine and Related Compounds

The ability of nicotine to stimulate and subsequently block transmission at ganglia has been known ever since the work of Langley and Dickinson (1889) Langley (1890) made use of its blocking action to find the actual anatomical location of various ganglia

The results of Feldberg and Vartianen (1934) using the perfused superior cervical ganglion of the cat, suggest that, when compared for ability to stimulate the ganglion and cause contracture of the nictitating membrane, nicotine is more active than acetylcholine, the equipotent molar ration being around 0 1 In the presence of eserine, however, much smaller amounts of acetylcholine are effective and the ratio is nearer to unity The relatively low stimulant activity of acetylcholine appears to be related to its susceptibility to destruction by cholinesterase in most test preparations Willey (1955) recorded an equipotent molar ratio for nicotine of 0 13 relative to

ncetyleholine compared for ability to raise the blood-pressure of the anaesthetized cat

Taylor (1951) suggested that nicotine was active as the univalent cation (VI 12), and although the effects of pH on its activity at ganglia have not been studied as they have at the neuromuscular junction (page 124), this seems to be likely to be correct. Nicotine monomethiodide (VI 13) is actually rather less active on the cat's superior cervical ganglion Barlow and Hamilton (1962) obtain n value of 4 9 for the equipotent ionic ratio of nicotine mono-methiodide relative to nicotine

R = H, nicotinium ion, VI 12
R = Me, monomethyl nicotinium ion, VI 13

$(CH_2)_n$—NR_2 and
—NR_2Me
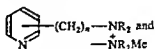
n = 1, VI 14,
n = 2, VI 15,

Barlow and Hamilton (1962) also studied a considerable number of analogues of nicotine, pyridylmethyl- and pyridyl-2-ethyldialkylamines and trialkylammonium salts (VI 14 and 15) These were tested mostly for ganglion-blocking activity on the cat's superior cervical ganglion, but some were also tested as ganglion stimulants In general, the equipotent molar ratios relative to nicotine were similar whether the compounds were compared as agonists or as blocking agents the one exception was β-pyridylmethyl methyldiethylammonium (VI 16), which was inactive as a stimulant, although it had feeble blocking properties It was thought that these compounds were, therefore, acting like nicotine, probably depolarising and then desensitizing the neurones in the ganglia Considerable blocking activity was shown by β-pyridyl 2-ethyltrimethylammonium (VI 17), (equipotent ionic ratio relative to nicotine, 0 088), β-pyridylmethylpyrrolidine (VI 18, ratio, 0 84) and β-pyridylmethyltrimethylammonium (VI 19, ratio, 0 98, ratio for stimulant activity, 0 70) The values for the equipotent ionic ratio of choline phenyl ether relative to nicotine on this preparation were 0 25 for stimulant activity and 0 66 for blocking activity
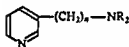
n = 1, NR₂ = ṄMeEt₂, VI 16,
n = 2, NR₂ = ṄMe₂ , VI 17,
n = 1, NR₂ = Ṅ◯ , VI 18
n = 1, NR₂ = ṄMe₃ , VI 19

The high activity of β-substituted pyridine deriva- tives was, in part, expected In these there will be a partial positive charge on the 2- and 4-positions of the pyridine ring, which can be compared with the partial positive charge on the ether oxygen of choline phenyl ether (Fig VI 3) The particularly high activity of the β-pyridyl-2-ethyl compound, however, was unexpected, although this compound can be compared with phenylethyltrimethylammonium, which is much more active than benzyltrimethylammonium (page 148) Another related compound with considerable activity is the substance leptodactyline (VI 20) Erspamer and Glässer (1960)
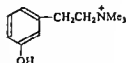
Leptodactyline, VI.20

Glässer (1960) compared this substance with nicotine and obtained equipotent molar ratios of 0 3 to 0 6 on the blood-pressure of the spinal cat, and 0 15 for blocking the response of the cat's nictitating membrane to preganglionic

$$Ph \leftarrow \overset{\delta+}{\underset{\delta+}{C}} - CH_2CH_2 - \overset{+}{N}Me_3$$
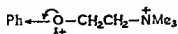
Choline phenyl ether (+I and +E effects)
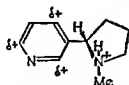
(-)-5-Nicotine

FIG VI 3   *Comparison of nicotine and choline phenyl ether*

stimulation Glasser and Pasini (1960) have studied the activity of a number of analogues of leptodactyline and some of the results are shown in Table VI 4 *m*-chlorophenyl-2-ethyltrimethylammonium is appreciably more active than Leptodactyline

TABLE VI 4

Ganglion Stimulant Activity of Some Analogues of Leptodactyline Equipotent Molar Ratios Relative to Leptodactyline for Ability to cause a Rise in Blood pressure in the Spinal Cat

$$R - \overbracket{} - CH_2CH_2 - \overset{+}{N}Me_3$$
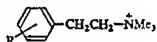
R =		S
m-Chloro-	0 50	+0 37
3 4-Dihydroxy-	0 60–0 96	−0 28
m-Hydroxy-	1 0	+0 12
H-	1 0–1 4	0
p-Hydroxy-	2 3–3 5	−0 38
m-Methoxy-	4 7–21	+0 11
m Methyl	6 1	−0 04

S is the Hammett constant for the analogous benzoic acids (see Table VI.2)

Glässer and Pasini (1960)

Dimethylphenylpiperazinium

Nicotine monomethiodide (Barlow and Dobson, 1955, Gillis and Lewis, 1956) and the analogues of nicotine studied by Barlow and Hamilton (1962) did not appear to differ greatly from nicotine itself in the ease with which their effects passed from stimulation to block If the situation at the ganglion can be expressed in the same way as at the neuromuscular junction by the relationship postulated by Katz and Thesleff (1957) involving the two types

of receptors, A and B (see page 134), it may be supposed that the ratio of the affinity constants, $K_B \cdot K_A$, is approximately the same for all these compounds, although the actual values of K_A and K_B will vary greatly from drug to drug Burn and Dale (1915), however, observed that although tetramethylammonium was quantitatively similar to nicotine in raising the blood-pressure of the spinal cat, it was less active as a ganglion-blocking agent, and in one substance in particular, dimethylphenylpiperazinium (VI 21 Chen, Portman, and Wickel, 1951) the separation of the two properties, stimulant and blocking, is very marked indeed Dimethyl-phenylpiperazinium will, in fact, cause a block in transmission in the ganglia of the guinea-pig ileum involved in the peristaltic reflex (Chen and Portman, 1954) and will also block transmission in the cat's superior cervical ganglion (Leach, 1957), but is generally regarded as a 'specific' ganglion stimulant Fakstorp and Pedersen (1954) describe the use of the compound (in preference to nicotine) for stimulating the ganglia of the intestine to produce regular control responses against which the actions of ganglion-blocking agents can be assayed Ling (1959) has shown that dimethylphenylpiperazinium is not only unusual in being 'specifically' a ganglion-stimulant but also is more active in stimulating the receptors in the adrenal medulla than those in sympathetic ganglia Ling, for instance, found an equipotent molar ratio of about 0 08 for dimethylphenylpiperazinium relative to nicotine in raising the blood-pressure of the spinal cat or causing contracture of the nictitating membrane, but when the adrenals were ligated the ratios were only 0 2 for the blood pressure and 0 8 for the nictitating membrane The effect of the drug on the blood pressure and nictitating membrane accordingly depends mostly upon the release of adrenaline and noradrenaline from the adrenal medulla

Chen and Parcell (1954) found that the piperidine analogue, 4 4-diphenyl-N-dimethylpiperidinium (VI 22) was equiactive when the compounds were compared for stimulant activity on the cat's superior cervical ganglion, on

R = Me, VI.22, R = iso-Pr, VI.23
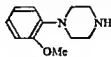
the bladder and on the blood pressure, but the isopropyl analogue, 4 4-diphenyl-N-methyl N-isopropylpiperidinium (VI 23) only blocked ganglia The compound 1-(o-methoxyphenyl)piperazine (VI 24) also lowers the blood-pressure, but this is not the consequence of an action at ganglia (Page, Wolford, and Corcoran, 1959)

Tropine Derivatives, *N-417*

Gyermek and Nador (1955) tested a number of tropine derivatives for ganglion-stimulant properties and found extremely high activity in p-phenyl-

benzyl (*p*-aminobenzoyl)-α-tropinium (*N-417*, VI 25) The equipotent molar ratio relative to nicotine was 0 017 on the cat's blood-pressure and 0 067 on the cat's superior cervical ganglion The *m*-phenylbenzyl group, *p* amino group and α-configuration of the tropine residue all appear to be important for activity If any of these is altered, activity drops markedly In view of the much greater effects of this compound on the blood-pressure than on the superior cervical ganglion it is possible that it may be particularly effective by releasing adrenaline and noradrenaline from the adrenal medulla
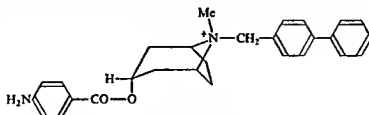
N-417, VI.25

Relationships Between Structure and Ganglion-stimulant Activity

The most active of the substances discussed appears to be *N-417* If it is assumed that the equipotent molar ratio for nicotine relative to acetylcholine on the cat's blood-pressure is 0 13, the ratio for *N-417* relative to acetylcholine should be around 0 002 The corresponding value for the *m*-bromophenyl ether of choline is 0 004 and for the phenyl ether itself, 0 012 β-Pyridyl-2-ethyltrimethylammonium may well be comparable in activity, but has only been tested for blocking activity on the superior cervical ganglion In this test the equipotent ionic ratio relative to nicotine was 0 088 (the equipotent molar ratio for *N-417* relative to nicotine was 0 067 on this preparation) and this suggests a ratio relative to acetylcholine of about 0 01 As a blocking agent the compound was appreciably more active than choline phenyl ether, for which the ratio relative to nicotine in these experiments was 0 66 Tiglycholine (equipotent molar ratio relative to acetylcholine, 0 03) and leptodactyline (equipotent molar ratio relative to nicotine, 0 3 to 0 6 for effects on blood pressure, 0 15 for block of the superior cervical ganglion) appear to be less active Dimethylphenylpiperazinium, dihydromurexine, trimethylacetylcholine, and *n* pentyl and *n*-hexyltrimethylammonium (all with equipotent molar ratios relative to acetylcholine of around 0 1) are less active still Although the figures for the equipotent molar ratios are based on different tests and should be treated with caution, they are probably reasonable enough to indicate the order of activity of the compounds

As already mentioned (page 151), Hey (1952) postulated that nicotine like stimulant activity depends upon the presence in the molecule of a partial positive charge a suitable distance from the cationic head This hypothesis has been most useful since it suggests new compounds which might be active, but it is also valuable because it is capable, to some extent, of being tested To obtain a quantitative estimate of the ability of substituents to attract or release electrons in substituted benzoyl esters of choline, Ormerod (1956)

ANTAGONISTS

(+)-Tubocurarine and Tetraethylammonium

The blocking action of (+) tubocurarine at ganglia is less marked than its action at the neuromuscular junction (page 134), but its ganglion-blocking activity is nevertheless quite high It may be of importance in poisoning with large doses of (+)-tubocurarine but cannot be made use of practically except as a tool in pharmacological experiments The first substance in which antagonism of the nicotine like actions of acetylcholine was confined to the sites in ganglia was tetraethylammonium (Burn and Dale, 1915) Although it is selective in blocking ganglia in doses which do not appreciably affect the neuromuscular junction, this substance is actually much less potent than (+) tubocurarine Acheson and Pereira (1946) found that the amount of tetraethylammonium needed to block the superior cervical ganglion of the cat was somewhere around twenty times the amount of (+)-tubocurarine

That ability to block conduction in ganglia does not necessarily have to be associated with ability to block the neuromuscular junction was also shown by the work of Bulbring and Depierre (1949) who studied the properties of a series of phenyl ethers of β hydroxyethyltriethylammonium, *F 2512*, *F 2557*, and *Gallamine Triethiodide* (VI 26, 27, and 28) The most active neuromuscular

$$ \underset{\underset{R''}{\overset{R'}{\Big|}}}{\bigodot} $$

R = OCH$_2$CH$_2$NEt$_3$ R = R' = H *F 2512*, VI.26,

R = R = OCH$_2$CH$_2$NEt$_3$ R' = H, *F 2557*, VI.27,

R = R = R' = OCH$_2$CH$_2$NEt$_3$ Gallamine Triethiodide, VI.28

blocking agent, *Gallamine Triethiodide* (see page 125), was actually the weakest ganglion blocking agent whereas *F 2512*, the weakest neuromuscular blocking agent, was the strongest blocking agent at the ganglion (the equipotent molar ratio relative to (+) tubocurarine chloride was between 4 0 and 10 0, Depierre, 1947)

Simple Onium Salts

Tetraethylammonium is not a particularly potent ganglionic-blocking agent, and although many simple onium salts have been found to have similar feeble activity, there are relatively few with marked ganglion blocking properties Winbury (1952) found the equipotent molar ratio of diethyldi*iso*-propylammonium (VI 29) relative to tetraethylammonium to be 0 077 on the superior cervical ganglion of the cat, for methylethyldi*iso*propylammonium (VI 30) the ratio was 0 14 Considerable activity was also shown by 2 6-dimethyl NN diethylpiperidinium (VI 31) for which Cook, Hambourger, and

Biaocbi (1950) obtained an equipotent molar ratio relative to tetraethyl-ammooium of 0 12 to 0 17, Winbury (1952) found the ratio to be 0 10 aod obtained the same value for the more complex structure, NN dimetbyl 1 benzyl 6 7 dihydroxy 1 2 3 4-tetrahydro isoquinolinium (VI 32)

R = Et, VI.29,
R = Me, VI 30

VI 31

VI 32
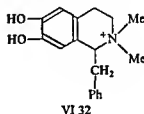
Trimetapban

Randall, Peterson, and Lebmaon (1949) found potent ganglion blocking activity in a ratber unusual type of compound, *Trimetaphan (Arfonad* VI 33), an analogue of biotin Part of the fall in blood pressure produced by this substance is due to its ability to cause the release of histamine from tissues

Trimetaphan VI 33
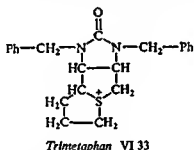
(Mitchell, Newmao, MacGillivray, and Clark, 1951) but the compound also, unquestionably, blocks conduction in ganglia as is seen from experiments with the cat s superior cervical gangltoo On this preparation the equipotent molar ratio for the (+)-isomer is around 0 01 relative to tetraethylammonium The (—) isomer is about half as active, and the compounds with one or both of the benzyl groups removed are only feebly active The effects of *Trimeta phan*, although intense, are extremely transient, presumably because the compound is rapidly broken down and/or excreted This short duration of action makes it a particularly suitable drug for use in surgery to reduce bleeding

Tropine Derivatives

Several tropine derivatives have been found to have ganglion blocking properties *Phenactropinum* (*Trophenium* VI 34) produces effects of short duration, and has an equipotent molar ratio relative to Hexamethonium (see below) of about 0 1 on the cat s superior cervical ganglion and 0 05 on the blood pressure (Robertson, Gillies and Spencer, 1957) It is slightly more active than *Trimetaphan* on the cat's superior cervical ganglion but the two compounds appear to be equiactive when used to reduce bleeding in

surgery (Robertson and Armitage, 1959) *Phenactropinium*, however, does
not cause the release of histamine
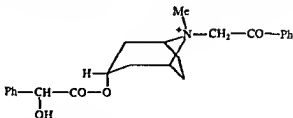
Phenactropinium, VI.34

Gyermek and Nador (1952, 1953) and Gyermek (1953, reviews by Gyermek
and Nador, 1957, and Nador, 1960) have studied the properties of a large
number of derivatives of tropine and some of the results are shown in Table
VI 5 Ganglion blocking activity appears to be influenced by the nature of
the alkylating group attached to the quaternary nitrogen atom and by the
nature of the esterifying acid Changes in these markedly alter the activity,
and yet the ester group is not indispensable, for the tropane derivative VI 35
is almost as active as the corresponding tropyl ester, *Gastropin* (VI 36) The
arrangement of the link with the tropane ring structure also influences activity
but, again, neither the α- nor the β-configuration can be regarded as essential
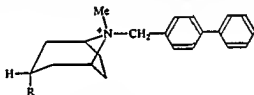
R = H, VI.35,
R = Tropyl, *Gastropin*, VI 36,
R = Mandelyl *N-310*, VI 37

for activity, for compounds of comparable activity are to be found in both
series (Table VI 6) Activity seems to be dependent primarily on the nature of
the groups attached to the quaternary nitrogen atom and it may perhaps be
that alterations in other parts of the molecule affect activity because some
substituents interfere with ability to fit the receptors rather than because other
substituents (e g mandelyl or *p* aminobenzoyl) positively assist the inter
action between the drug and the receptors

A comparison between these compounds and *Phenactropinium* does not
seem to have been made -- the available results suggest that the latter is more
active than *N-310* (VI 37) or *Gastropin* -- but with all these compounds it
seems that activity is associated with the presence of electron withdrawing
groups attached to the quaternary nitrogen atom *Gastropin* which has some
blocking activity at the postganglionic parasympathetic receptors on smooth
muscle as well as ganglion blocking activity (Gyermek and Nador, 1957),
has been used for the treatment of gastric and duodenal ulcers

TABLE VI 5

Ganglion blocking Activity of Tropine Derivatives

R₁	R₂	Cat's superior cervical ganglion equipotent molar ratios relative to	
		Tetraethyl ammonium	Hexa methonium
H	Tropyl (*atropine*)	1 7	44
Me	Tropyl (*Eumydrine*)	0 14	3 6
Me	Mandelyl (*Novatropine*)	0 13	3 3
PhCH₂	Benzoyl	0 17	4 4
PhCH₂	Benzoyl (β)	1 0	26
PhCH₂	p-aminobenzoyl	0 061	1 6
Cl-◯-CH₃	p-aminobenzoyl	0 020	0·51
Cl-◯-CH₃	p-nitrobenzoyl	0 053	1 4
◯-CH₃	mandelyl	0 16	4 1
o-Bromobenzyl	mandelyl	0·098	2 5
m-Bromobenzyl	mandelyl	0·059	1 5
p-Bromobenzyl	mandelyl	0 021	0 54
p-Nitrobenzyl	mandelyl	0 027	0 69
p-Methoxybenzyl	mandelyl	0 055	1 4
Ph-◯-CH₂	mandelyl (*N 310* VI 37)	0 010	0 26
PhCH₂	phenylacetyl	0 10	2 6
p-Bromobenzyl	phenylacetyl	0 046	1 2
Ph-◯-CH₂	phenylacetyl	0 041	1 1
Ph-◯-CH₂	diphenylacetyl	0 036	0 92
Ph-◯-CH₂	Tropyl (*Gastropin* VI 36)	0 016	0 41
Ph-◯-CH₂	H (VI 35)	0 019	0 49
Hexamethonium		0 039	1 0

β indicates that the benzoyl group is *cis* to the ring nitrogen i e equatorial and not axial as in the other (α) compounds

Gyermek and Nador (1953 1957) *Nador* (1960)

TABLE VI 6

Effect of Position of Acyl Group on Activity

	Cat's superior cervical ganglion equipotent molar ratios relative to tetraethyl ammonium	
	α	β
R =		
H	7 5	7 5
Me	0 27	2 7
Et	0 20	0 089
n Pr	0·20	0 095
n Bu	0 092	0 12
PhCH₂	0 17	1 0

Gyermek (1953)

Other Ganglion-Blocking Onium Salts Used in the Treatment of Gastric and Duodenal Ulcers

The use of atropine to reduce gastric secretion and the motility of the stomach and gut is a logical application of its ability to block the actions of acetyl choline at the postganglionic parasympathetic receptors (Chapter VII) The treatment has certain disadvantages in particular, the atropine is absorbed through the gut wall and, particularly if treatment is lengthy, produces effects on the body as a whole Many substances have been tested as substitutes for atropine and among these a number of onium salts It has generally been found (page 216) that quaternization increases ability to antagonize *the action* of acetylcholine at the postganglionic parasympathetic receptors and, further, the onium group is fully ionized and therefore the compound is not likely
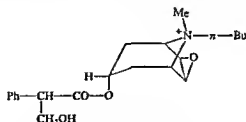
Buscopan VI 38

to be soluble in fat Consequently the general absorption of these compounds should be much less than that of tertiary bases It may be questioned how far it is possible to achieve sufficient absorption into the gut wall for an action on the nerve-plexuses within it without also obtaining sufficient general absorption to affect the rest of the autonomic nervous system Nevertheless many compounds of this type have been tried and some, like *Gastropin* possess considerable ganglion blocking activity It is possible that this may, in certain

circumstances, be more important than the blocking activity of the compounds at the postganglionic parasympathetic receptors. *Buscopan* (N-*n* butyl-hyoscinium, VI 38) has an equipotent molar ratio relative to Hexamethonium of 2 0 on the cat's superior cervical ganglion, and for *Methanthelinium* (*Ban thine*, VI 39) the ratio is 30 (Gyermek and Nador, 1957). Bainbridge and
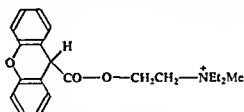
Methanthelinium VI.39

Brown (1960) have studied the action of some of these substances on the cat's superior cervical ganglion using as a measure of the block the reduction of the size of the action potential in the post ganglionic fibre in response to preganglionic stimulation. The results (Table VI 7) are rather different from

TABLE VI 7

Activity of Drugs in Reducing Transmission in the Cat's Superior Cervical Ganglion

	Equipotent molar ratios relative to Hexamethonium*
Pentolinium	0 15
Methylatropinium (*Eumydrine*)	0 45
Mecamylamine	1·4
Propantheline	1 8
Methantheline	2 0
Atropine	4 0
Tetraethylammonium	4 4

* Obtained by comparing the doses (injected into the femoral vein) which reduced by 50 per cent the height of the postganglionic action potential in response to preganglionic stimulation

Bainbridge and Brown (1960)

those obtained by Gyermek and Nador (see also Table VI 5), possibly because of differences in the conditions of stimulation (see page 182). The activity of these compounds at parasympathetic ganglia is difficult to assess.

Bis-onium Salts. Pentamethonium and Hexamethonium

Although some *mono* onium salts with considerable ganglion blocking activity were known, the development of drugs with sufficient sustained activity and specificity for clinical use followed upon the discovery by Paton and Zaimis (1949, 1951) of high ganglion blocking activity in *bis* onium salts, in particular in Pentamethonium (**VI 40**) and Hexamethonium (**VI 41**). This

optimal for activity because the effects of a particular group on activity depend upon the number of methylene groups in the chain Although penta methylene *bis* N methylpyrrolidinium (Pentolinium VI 44) is the most

Pentolinium, VI 44

active of the compounds in Table VI 9, at least as far as the sympathetic ganglion is concerned, in the tetramethylene compounds it is the *bis* N methyl piperidinium compound which is most active The N methylpyrrolidinium unit can be regarded as conferring activity, but it is not as effective as the N methylpiperidinium group at the shorter chain length One generalization which may, perhaps, be drawn from the results is that with the larger onium groups, such as methyldiethylammonium, N methylpiperidinium and N methylmorpholinium, the activity is much less dependent upon the chain length than it is with the smaller onium groups, such as trimethylammonium
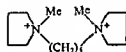
TABLE VI 9

Ganglion Blocking Activity of Analogues of Hexamethonium

$$\overset{+}{R_3N}-(CH_2)_n-\overset{+}{N}R_3$$

Equipotent Molar Ratios Relative to Hexamethonium on (a) Cat's Superior Cervical Ganglion and (b) the Peristaltic Reflex of the Guinea pig Ileum

n =	$R_3 = Me_3$ (a)	(b)	Me_2Et (a)	(b)	$MeEt_2$ (a)	(b)	Et_3 (a)	(b)
4	110	22	10	40	093	37	17	17
5	16	14	065	065	071	89	12	89
6	1	1	062	046	1	086	>16	>40
7	80	32	89	30	55	42	39	>39

n =	$R_3N = Me_2n\ PrN$ (a)	(b)	$Me\ isoPrN$ (a)	(b)	Me_2HN (a)	Me_3S (a)	$MeEtHN$ (a)	$MeEt_2S$ (a)	Et_2HN (a)	Et_3S (a)
6	>17	>17	57	86	120	20	82	35	71	19

n =	$R_3N =$ (pyrrolidinium) $\overset{+}{N}-Me$ (a)	(piperidinium) $\overset{+}{N}-Me$ (a)	(morpholinium) $\overset{+}{N}-Me$ (a)	(b)
4	43	089	15	25
5	084	094	015	049
6	067	084	029	087
7	082	22		

Wien and Mason (1951) Wien Mason Edge and Langston (1952) Barlow and Vane (1956)

Effects of Altering the Polymethylene Chain

Wien and Mason (1953) investigated the effects of introducing a benzene ring into the chain linking the two onium groups Considerable activity is shown by some of the compounds (Table VI 10), particularly by phenylethane-*p-ω-bis*-onium salts, some of which are as powerful as Pentolinium

TABLE VI 10
Activity of Phenylalkane bis onium Salts
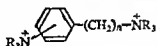
$$R_3\overset{+}{N}\text{—}\langle\langle\rangle\rangle\text{—}(CH_2)_n\text{—}\overset{+}{N}R_3$$

	Equipotent molar ratios relative to Hexamethonium on (a) cat's superior cervical ganglion, (b) peristaltic reflex of guinea pig ileum							
	R₃ = *m*-Me₂		*p* Me₂		*p*-Me₂Et		*p* MeEt₂	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
n = 1	>160	>160	160	>16	—	—	—	—
2	0 51	0 61	0 25	0 30	0 22	0 29	0 38	0 68
3	0 74	1 5	2 9	1 5	t 4	0 70	4 4	t 3
4	0 72	0 72	—	—	—	—	—	—

Wien and Mason (1953)

The bridge linking the onium groups can also contain a reduced ring system Colville and Fanelli (1956) studied derivatives of 4-(2-amino-ethyl)piperidine, and the activity of some of these, e g 4-(2-N pyrrolidino-ethyl)-N methylpiperidine *bis*-etho and *bis*-metho salts (VI 45 and 46),

$$\left[\begin{array}{c}\overset{+}{N}\text{—}CH_2CH_2\text{—}\langle\langle\rangle\rangle\text{—}\overset{+}{N}\overset{Me}{\underset{R}{}}\end{array}\right]$$
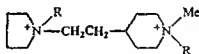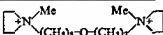
R = Et, VI 45,
R = Me, VI 46

appeared to be comparable with that of Hexamethonium on the superior cervical ganglion of the cat

Compounds in which a methylene group is replaced by an ether oxygen were studied in detail by Fakstorp and Pedersen (1954, 1957), Fakstorp, Poulsen, Richter, and Schilling (1955), and Fakstorp, Pedersen, Poulson, and Schilling (1957) With an ether link in the molecule it is easy to prepare asymmetrical *bis*-onium salts, and the results, some of which are shown in Table VI 11, suggest that such asymmetrical compounds are more active *than either of the corresponding symmetrical analogues* The most active compounds are those with chain lengths similar to those of Pentamethonium and Hexamethonium The ether link appears to contribute slightly to activity when the chain length is comparable with that of Hexamethonium,

TABLE VI.II

Ganglion Blocking Properties of Ether-linked bis-onium Salts

$R_3\overset{+}{N}$–$(CH_2)_n$–O–$(CH_2)_m$–$\overset{+}{N}R'_3$	Equipotent molar ratios relative to Hexamethonium	
	Cat superior cervical ganglion	Ileum stimulated with dimethyl phenyl piperazinium
1. *Effect of chain length*		
$R_3 = Me_3$ $R'_3 = Me_3$		
$n = 2$ $m = 2$	2 0	3 1
3 (VI 47) 2	0 54	0 97
3 3	7 1	49
2 *Effect of onium groups*		
$n = 2$ $m = 2$		
$R_3 = Et_3$ $R'_3 = MeEt_2$	0 45	1 4
Et_3 Me_2Et	0 59	0 70
Et_3 Me_3	very large	
Et_3 Et_3	13 1	13 1
$MeEt_2$ $MeEt_2$	2 0	2 3
Me_2Et Me_2Et	2 9	4 0
$Et_2\,n\text{-}Pr$ Me_2Et	1 1	0 98
$Et_2\,n\text{-}Bu$ Me_2Et	0 77	0 44
$n = 3$ $m = 2$		
Me_2Et Me_2Et	0 60	1 9
$MeEt_2$ $MeEt_2$	1 5	1 2
Me_3 $MeEt_2$	0 58	0 35
Me_2Et Et_3	0 39	0 56

$R_3\overset{+}{N}$–$(CH_2)_n$–X–$(CH_2)_m$–$\overset{+}{N}R'_3$						
$R_3 =$	$n =$	$X =$	$m =$	$R'_3 =$		
Me_3	2	–O–	2	Me_3	2 0	3 1
Me_3	2	–S–	2	Me_3	1 5	1 3
Me_3	2	–S–S–	2	Me_3	1 8	1 4
Et_3	2	–O–	2	Me_2Et	0 59	0 70
Et_3	2	–S–	2	Me_2Et	0 55	0·37
$\left[\quad\right]\overset{+}{N}\!\!-\!\!Me \quad Me\!\!-\!\!\overset{+}{N}\left[\quad\right]$ $(CH_2)_3$–O–$(CH_2)_3$					1 0	0·78

Fakstorp, Pedersen, Poulsen, and Schilling (1957); Fakstorp and Pedersen (1957)

the equipotent molar ratio for compound VI 47 relative to Hexamethonium on the superior cervical ganglion being 0 54 At shorter chain lengths, however, the ether link appears to reduce activity, the ether analogue of Pentamethonium (equipotent molar ratio relative to Hexamethonium on the

$$Me_3\overset{+}{N}-(CH_2)_5-O-(CH_2)_2-\overset{+}{N}Me_3$$

VI 47
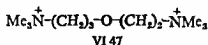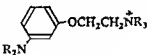
superior cervical ganglion, 2 0) being less active than Pentamethonium (ratio, 1 3), and the ether analogue of Pentolinium (ratio, 1 0) being less active than Pentolinium (ratio, 0 14) The thioethers seem to be more active than the corresponding ethers, but it is not possible to say how far this might be due to an interaction between the sulphur atom and a receptor group, and how far it might be due to the greater separation of the onium groups in these compounds

The results obtained with these compounds are interesting not only because *the effects on activity of different substituents in the onium groups vary with* the length of the polymethylene chain but also because they indicate that activity at parasympathetic ganglia is not necessarily associated with activity at sympathetic ganglia

Fakstorp and Pedersen (1958) have also studied the activity of *bis* onium salts which are derivatives of choline phenyl ether and are, therefore, analogues of some of the compounds studied by Wien and Mason (1953) The results (Table VI 12) are interesting for a number of reasons Some of the compounds containing one tertiary amino group have activity comparable with that of the *bis* quaternary salts, and the effects on activity of changes in

TABLE VI.12

Ganglion Blocking Activity of Substituted Phenyl Ethers of Choline

R_2N⟨benzene ring⟩$-OCH_2CH_2\overset{+}{N}R_3$		Equipotent molar ratios relative to Hexamethonium	
		(a)	(b)
$R_2 =$	$R_3 =$		
Et_2	Me_3	1 6	2 9
Et_2	Me_2Et	2 0	0 81
Et_2	Et_3	1 2	0 71
Me_2	Me_3	2 4	4 0
Me_2	Me_3 (*p*-isomer)	1 8	3 0
Me_2Et	Me_2Et	1 5	3 8
$MeEt_2$	$MeEt_2$	>1 4	4 9
Me_2	$MeEt_2$	2 6	5 1
$MeEt_2$	Me_3	1 4	0 98

(*a*) Cat s superior cervical ganglion
(*b*) Guinea pig ileum stimulated with dimethylphenyl piperazinium.

Fakstorp and Pedersen (1958)

the substituents on the onium nitrogen attached to the benzene ring are not the same as when the same changes are made to the onium nitrogen at the end of the ethylene chain. The *m* linked bis trimethylammonium compound (VI 48) does not appear to be ns active as the compound in which the ether
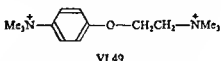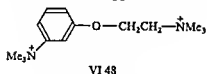
oxygen is replaced by a methylene group (Table VI 10), but the *p* linked bis trimethylammonium compound (VI 49) appears to be at least as active as *p* phenyl n propane-α ω bis trimethylammonium

Azamethonium (*Pendiomide*, VI 50, Bein and Meier, 1950) This compound

$$EtMe_2\overset{+}{N}-CH_2CH_2-\overset{Me}{\overset{|}{N}}-CH_2CH_2-\overset{+}{N}Me_2Et$$
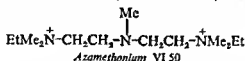
Azamethonium VI 50

contains a methylamino group in place of the middle methylene group in Pentamethonium and is a *bis* ethyldimethylammonium salt. The *bis* trimethylammonium analogue is much less active, and even *Azamethonium* itself is not as active as Hexamethonium (Blackman *et al*, 1956, recorded an equipotent molar ratio of 1 2 for their effects on blood pressure in rabbits, Table VI 13)

TABLE VI 13
Effects of Some Ganglion blocking Agents in Lowering the Blood pressure

	Equipotent molar ratios relative to Hexamethonium	
	In conscious rabbits	In Man*
Chlorisondamine	0 1	0 06
Pentolinium	0 2	0 13
Hexamethylene-*bis*-ethyldimethylammonium (VI)	0 8	0·52
Azamethonium	1 2	1 0

* By injection based on the value of 0 13 for Pentolinium relative to Hexamethonium calculated from the results of Smirk (1953)

Blackman Fastier, Patel and Wong (1956)

Compounds in which part of the chain is an ester link have been studied by Schueler and Keasling (1954) and by Hidalgo, Wilken, and Seeberg (1959) The simple *bis* trimethylammonium compound, VI 51, was a ganglion

$$Me_3\overset{+}{N}-CH_2CH_2-CO-O-CH_2CH_2\overset{+}{N}Me_3$$

VI 51
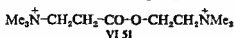
blocking agent, but the activity of this compound and of others with different substituents on the onium groups does not appear to be very striking The

ester link definitely appears to reduce blocking activity, but methyl substituents in the chain, β- to an onium group (i e esters of β-methylcholine), appear to increase activity somewhat

Asymmetrical *bis*-Onium Salts

Plummer *et al* (1955) studied the properties of a number of asymmetrical *bis* onium salts in which one onium group was a substituted *isoindolinium* group and the other an aliphatic onium group (VI 52) In this series activity

$$R \begin{array}{c} R \\ R \end{array} \begin{array}{c} R \\ N^+ \\ (CH_2)_n - \overset{+}{N}R_2'R'' \end{array}$$
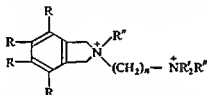
VI 52,
R = H, n = 2, R′ = R″ = Me, VI 53,
R = Cl, n = 2, R′ = R″ = Me *Chlorisondamine*, VI 54,
R = Cl, n = 2, R = Et, R″ = Me, VI 55

was greatest in compounds with a short polymethylene chain 2 (2′-dimethylaminoethyl) *isoindoline bis* methiodide (VI 53), for instance, had an equipotent molar ratio relative to Hexamethonium of 0 70 on the cat's superior cervical ganglion Activity was greatly increased by substitution of halogen in the benzene ring, for 2-(2′-dimethylaminoethyl)-4 5 6 7-tetrachloro-*isoindoline bis* methchloride (VI 54, *Chlorisondamine*, *Ecolid*) the ratio was 0 14 Activity was apparently not increased, however, by the replacement of methyl groups by ethyl, for 2-(2 -diethylaminoethyl) 4 5 6 7-tetrachloro-*isoindoline bis*-methiodide (VI 55) the ratio was 0 48

Adamson, Billinghurst, and Green (1956) and Billinghurst (1956) and Green (1956) have studied a group of compounds which resemble *Chlorisondamine* in having only a short polymethylene chain and a relatively large group at one end Some of these substances appear to be very highly active indeed when compared with *Chlorisondamine* for ability to cause mydriasis The

$$\begin{array}{c} Ph \quad CN \\ Ph-C-(CH_2)_n-\overset{Me}{\underset{Me}{N}}-CH_2CH_2-\overset{+}{N} \end{array} O$$
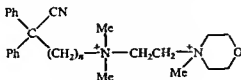
n = 5, 356 C 54, VI 56
n = 4, 139 C 55, VI 57

results of clinical tests were not so impressive, but *356 C 54* (VI 56) and *139 C 55* (VI 57) appear, nevertheless, to be at least as active as, and probably more active than *Chlorisondamine* (Table VI 14)

Compounds in which the large group at one end of the chain is a tropine derivative have been studied by Lape, Fort, and Hoppe (1956) Some of these (Table VI 15) are more active than Hexamethonium on the cat's

TABLE VI.14

Mydriatic Activity of Asymmetrical bis-onium Salts 139 C 55 and 356 C 54

	Equipotent molar ratios relative to *Chlorisondamine*	Clinical dose used for treating high blood-pressure (μм/kg)
356 C 54	1 0	0 12–1 2
139 C 55	0 7	0·06–0 6
Chlorisondamine . . .	1 0	0 09–0 9
Pentolinium	7 0	0 2 –2 0

The lower figure in the range of doses used clinically may give some idea of the equipotent molar ratio relative to Hexamethonium on the blood-pressure. the ratio for Pentolinium relative to Hexamethonium is around 0 2 and for chlorisondamine around 0 1 (see Tables VI.9 and 13)
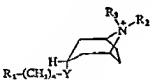
Green (1956).

superior cervical ganglion, and one or two compounds were very active indeed in blocking the carotid sinus reflex. This latter activity, however, appears to be due to a central action of the drugs rather than to their ganglion-blocking properties.

For further examples of asymmetrical *bis*-onium salts with a large group at one end see the reviews by Cavallito and Gray (1960) and Nador (1960).

TABLE VI 15

Activity of Some Asymmetrical bis-onium Salts Derived from Tropine on the Cat's Superior Cervical Ganglion

$R_1 =$	n	Y	R_2	R_3	Equipotent molar ratio relative to Hexamethonium
[structure] Me	3	NH	Me	Me	0 27
[structure] Me	2	NH	Me	Me	0 42
Me₂EtN	2	O	Et	Me	0 41
MeEt₂N	2	NH	Me	$-CH_2$ [structure]	0 27
MeEt₂N	2	NMe	Me	$-CH_2$ [structure]	1 2

The equipotent molar ratios for the last two compounds relative to Hexamethonium in the carotid sinus test were 0·096 and 0 017 respectively.

Lape, Fort, and Hoppe (1956)

Ganglion-blocking Compounds Which are Secondary or Tertiary Bases

Although powerful ganglion blocking activity was first found among *bis-onium* salts which are permanent cations, such as Hexamethonium, the results do not suggest that activity is confined to this one class of compound For use in the treatment of high blood pressure it is, in fact, desirable to avoid onium salts because they are irregularly and unpredictably absorbed when taken by mouth The oral dose of Hexamethonium, for instance, is about ten times the dose by injection Further, even when an onium salt reaches the blood stream it is likely to be rapidly excreted, for it will be filtered by the glomerulus of the kidney, but is unlikely to be reabsorbed into the blood stream in the tubule Considerable attention has, therefore, been given to ganglion blocking agents which are not onium salts

Plummer, Schneider, and Barret (1954) found considerable ganglion-blocking activity in a group of tetramines which can be regarded as tertiary derivatives of *Azamethonium* The most active member of the group, VI 58,

$$Et_2N \underset{\underset{\underset{NEt_2}{(CH_2)_3}}{\overset{(CH_2)_2}{\big|}}}{\overset{(CH_2)_2}{N}} NEt_2$$

VI 58
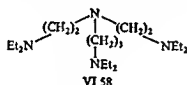
had, in fact, blocking activity on the cat's superior cervical ganglion comparable with that of *Azamethonium* and Hexamethonium The next development, however, was an apparently unrelated compound, *Mecamylamine* (*Inversine*, VI 59, Baer, Paulson, Russo, and Beyer, 1956) In terms of the dose required

and/or mirror images of either

R = Me, *Mecamylamine*, VI 59
R = H, VI 60
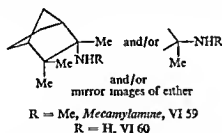
to produce a particular degree of block, this compound was at least as active as Hexamethonium (Stone, Torchiana, Navarro, and Beyer, 1956), but its effects persisted for from three to five times the length of time In spite of this, and in spite of the lack of any obvious resemblance to Hexamethonium, *Mecamylamine* appeared to be acting in essentially the same way, blocking transmission in ganglia

Lee, Wragg, Corne, Edge, and Reading (1958) observed that the reduction of crude 3 nitro *iso*camphane with lithium aluminium hydride gave not only the primary amine, *nor*-Mecamylamine (VI 60), but also some isomeric secondary amines These were thought to be the fused six- and seven membered

ring substances, VI 61 and 62, and it was observed that these, and their derivatives, also possessed ganglion-blocking activity Corne and Edge (1958) accordingly studied the simpler six membered ring structures, the alkylated piperidines

VI 61   VI 62

Spinks and Young (1958), working on the ganglion blocking activity of aliphatic amines, observed some activity in compounds such as N dimethyl (1 1 3 3 tetramethyl) *n* butylamine (VI 63), N ethyl (1 1 3 3 tetra methyl) *n* butylamine (VI 64), and N-ethyl *tert*butylamine (VI 65) They

$$Me\text{-}\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{C}}\text{-}CH_2\text{-}\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{C}}\text{-}NRR \qquad Me\text{-}\underset{\underset{McH}{|}}{\overset{\overset{Me}{|}}{C}}\text{-}N\text{-}R$$
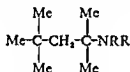
R = R = Me, VI 63
R = H R = Et VI 64

R = Et VI 65
R = -CMe₃ VI 66

speculated that high activity might be found in di *tert*butylamine (VI 66) but this compound is difficult to synthesize and is also unstable, so they decided to investigate the alkylated piperidines, which can be regarded as being derivatives of di *tert*butylamine

Both groups independently discovered the high activity of 1 2 2 6 6-pentamethylpiperidine (*Pempidine*, VI 67) and of compounds related to it

Pempidine, VI 67

Some of the results are shown in Tables VI 16 and 17 Because of the long duration of action of these compounds the comparisons of their activity relative to Hexamethonium cannot be particularly accurate Spinks, Young, Farrington, and Dunlop (1958) used *Mecamylamine* as the standard, and as the dose of this compound required to produce a particular degree of block was, in their experiments, about

the same as, or slightly less than, the dose of Hexamethonium, the values of the equipotent molar ratios of the analogues of *Pempidine* can be taken as being approximately the same as the ratios relative to Hexamethonium Estimates of the value for *Pempidine* vary considerably, Corne and Edge (1958) obtained the value 0 96, Spinks, Young, Farrington, and Dunlop (1958) obtained the value 0 43 This emphasizes the difficulties inherent in comparing long acting compounds of this type On the (parasympathetic) ciliary ganglion, Corne and Edge obtained the value 0 32 to 0 65 for Pempidine relative to Hexamethonium and the effects lasted from one and a half to ten times as long

Activity appears to depend greatly on the number of methyl groups adja-

TABLE VI 16

Ganglion Blocking Activity of Analogues of Mecamylamine

		Equipotent molar ratios relative to Hexamethonium on the cat's superior cervical ganglion
	R = H (VI 60)	8 8
	= Me (*Mecamylamine*)	0 98
	R = Me	5 8
	= Et	5 3
	R = H (VI 61)	0 98
	= Me	1 2
	R = H (VI 62)	1 7
	= Me	3 0
	(*Pempidine*)	0 96

Corne and Edge (1958); *Edge, Corne, Lee, and Wragg* (1960)

TABLE VI 17

Ganglion Blocking Activity of Analogues of Pempidine

	Equipotent molar ratios relative to *Mecamylamine* on cat's superior cervical ganglion
2 2 6-Trimethyl piperidine	3 3
2 2 6 6-Tetramethylpiperidine	0 59
2 2 6 6-Tetramethyl-*N*-methyl piperidine	0 43
2 2 6 6-Tetramethyl-N-ethyl piperidine	0 33
2 2 6 6-Tetramethyl N n propyl piperidine	0 46
2 2 6 6-Tetramethyl-N-*n* butyl piperidine	0 57
2 2 6 6-Tetramethyl N-allyl piperidine	0 77

Note – The ratio for *Mecamylamine* relative to Hexamethonium in this work was 0 89 (Corne and Edge obtained the value 0 98), consequently the figures on this table are approximately the same as the ratios relative to Hexamethonium Note, however, that in these experiments the ratio for Hexamethonium relative to tetraethylammonium was 0 34 (cf 0 028 in Table VI 8)

Spinks, Young, Farrington, and Dunlop (1958)

cent to the amino nitrogen atom The loss of a single methyl group in either *Mecamylamine* or *Pempidine* reduces activity very markedly (Tables VI 16 and 17, qualitative evidence for this is also to be found in the work of Ruhinstein, Pedersen, Fakstorp, and Rønnov-Jessen, 1958) An increase in the degree of substitution on the amino nitrogen atom, on the other hand, appears to increase activity The ethyl and *n* propyl analogues of *Pempidine* are more active than Pempidine itself, and *Dimecamin* (VI 68, Vejdelek and Protiva,

Me

Dimecamin VI.68

Penhexamine VI 69

1958) appears to be slightly more active than *Mecamylamine* The structure of the saturated ring does not seem to be particularly important Protiva, Rajsner, Trcka, Vanecek, and Vejdelek (1959) found comparable ganglion blocking activity in N N 1 2 2 pentamethyl)*cyclo*hexylamine (*Penhexamine*, VI 69), and Vejdelek and Trcka (1959) observed similar high activity in compounds such as N dimethyl (1 1 2 2 tetramethyl) *n* propylamine (*Penbutamine*, VI 70) which contains no ring at all The equipotent molar

$$CH_3-C-C-NMe_2$$
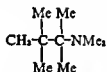
Penbutamine, VI 70

ratios for *Dimecamin*, *Penhexamine*, and *Penbutamine* on the cat's superior cervical ganglion relative to *Mecamylamine* appear to lie between 0 5 and 1 0 It is interesting to compare this activity of *Penbutamine* with the ratio of about 10 relative to *Mecamylamine* obtained by Spinks, Young, Farrington, and Dunlop for N dimethyl (1 1 3 3 tetramethyl)-*n* butylamine (VI 63) on the same preparation

Although the effects of these non quaternary compounds on the blood pressure could he due to an action on the central nervous system, since they should readily penetrate the blood hrain harrier, there is evidence that they do not act in this way, nor hy blocking conduction in nerve trunks Substances such as *Mecamylamine* have some direct effects on muscle (Bennett, Tyler and Zaimis, 1957), hut only in relatively high concentrations, for they are not particularly active at the neuromuscular junction It seems that these substances act at ganglia in a manner similar to that of Hexamethonium Corne and Edge (1958), for instance, found that *Pempidine* had no ganglion stimu lant activity and did not cause contracture of the frog rectus preparation The quaternary analogues do not appear tn have been studied in detail, hut Spinks, Young, Farrington, and Dunlop (1958) state 'Quaternization of the

tertiary amines caused loss of persistence some quaternary compounds were highly active, but their action was rapid in onset and transient'

The differences between the pharmacological properties of the quaternary and non-quaternary ganglion blocking agents appear to be ascribable only to physical differences in their relative ease of penetration

While it is acceptable in theory, and experimentally observed in fact, that secondary and tertiary bases can penetrate membranes by virtue of their ability to exist partly in an unionized fat soluble form, there are certain difficulties Blackman and Ray (1964) have pointed out that both *Mecamyl-amine* and *Pempidine* are very strong bases with pK_a values between 11 and 12 At body pH less than 1 part in 10,000 will be present in the unionized form, and this seems a very small fraction to account for the penetration of these compounds through membranes, even though the rate of penetration is slow Clearly more information is needed about the ways in which molecules of all kinds penetrate membranes

Specificity of Drugs for Particular Ganglia

It would be very useful to know what factors favour ability to block one type of ganglion rather than another The use of ganglion blocking agents in the treatment of high blood-pressure depends upon their ability to block sympathetic ganglia, and it is the blocking activity at parasympathetic ganglia which leads to the undesirable side effects of the drugs Some attempts have been made to measure activity at both types of ganglia, but most ganglion-blocking agents appear to block sympathetic and parasympathetic ganglia to about the same extent Although there are some substances, e g among the ethers in Table VI 11, which appear to block one type of ganglion rather than another, it must be remembered that estimates of ability to block para-sympathetic ganglia obtained using the guinea pig ileum stimulated by dimethylphenylpiperazinium cannot be very reliable

There do, however, appear to be detectable differences between the re ceptors in the ganglia and those in the adrenal medulla The ability of some drugs to stimulate the adrenal medulla more than sympathetic ganglia has already been commented on in connexion with the actions of drugs such as the phenyl ethers of choline and dimethylphenylpiperazinium (page 156) Gardier, Abreu, Richards, and Herrlich (1960) have found that N N di-isopropyl-N -isopentyl N'-diethylamino-ethyl urea (VI 71) appears to block

$$\text{isoPr} \diagdown \text{N-C-N} \diagup \text{CH}_2\text{CH}_2\text{CHMe}_2 \diagdown \text{CH}_2\text{CH}_2\text{-NEt}_2$$

VI 71
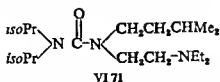
cholinergic transmission in the adrenal medulla, but has only a feeble action at sympathetic ganglia If this substance really acts as a blocking agent at the cholinergic receptors in the adrenal medulla, these must be distinctly different in structure from the receptors in the ganglia

Relationships Between Structure and Ganglion-blocking Activity

Two factors must be considered, how far changes in structure alter efficacy and how far they alter affinity. The relationships between structure and stimulant activity at ganglia have already been discussed (page 157), and stimulant activity indicates the presence of efficacy, though not in any quantitative fashion. Ing (1956) suggested that 'in any ganglionic stimulator which contains one methylated onium atom replacement of all the methyl groups by heavier groups will convert the compound into a purely blocking agent' and also that 'the more powerful a stimulator the methylated onium salt is, the more powerful a blocking agent the analogous ethylated onium salt will be'.

These suggestions appear to be partly true, but require some modification. The first observation seems to be borne out by experience. All known ganglion stimulants possess one or more methyl groups attached to the onium atom, many compounds possess three, and any increase in the size of the groups attached to the onium atom leads to loss of stimulant activity. These compounds with no efficacy derived from powerful stimulants might well be expected to be powerful blocking agents, provided the change which has destroyed efficacy has not also destroyed affinity. Since the affinity depends to a considerable extent on the part of the molecule other than the onium group, the substitution of relatively small groups, such as ethyl, in order to destroy efficacy, should result in a molecule with high affinity and no efficacy which should be a competitive ganglion blocking agent. The substitution of larger groups on the onium atom might destroy affinity as well as efficacy, but this does not necessarily follow, because these groups themselves might contribute to the affinity of the molecule.

These considerations however fail to take account of the molecules which both stimulate and block. As at the neuromuscular junction, not enough is known yet about the conditions connecting depolarization and desensitization, if these are, in fact, connected. There does not appear to be any reason why a molecule should not be found with very high affinity for the type B receptors to the ganglia (page 135) which would be a potent ganglion blocking agent of a type not considered by Ing.

The relationships between structure and ganglion blocking activity of the type assumed to be competitive have been discussed by Gill and Ing (1958) and by Gill (1959). There is considerable evidence that the attachment of *bis* onium salts to the receptors in ganglia involves two points, the receptor group binding one cationic head and an 'anchoring' group binding the other. These groups are considered not to be identical and this view is consistent with the results of the experiments with asymmetrical *bis* onium salts. In these and more especially, in other types of molecule, attachment by van der Waals' forces at points intermediate between the two receptor groups may be very important. With the long polymethylene *bis* triethylammonium salts, for instance, the gradual increase in activity with chain length is consistent with attachment of one onium group at the receptors and van der Waals'

forces along the length of the chain Ganglion-blocking activity appears to be associated to a very considerable extent, e g in substances like *Mecamylamine* and *Pempidine*, with the presence of large groups close to the onium nitrogen atom (even in the non-quaternary compounds the amino group of most molecules will carry a proton) The steric hindrance, for instance, is sufficient for it to be quite difficult to methylate *Dimecamin* For this reason it would seem likely that the attachment of most *mono*-onium ganglion blocking agents involves van der Waals' forces in the region close to the receptor-group binding the onium nitrogen, rather than additional receptor groups Possibly the molecules even act by virtue of their hulk in this region.
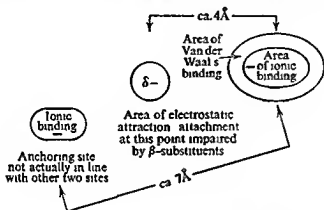
FIG VI 6   *Hypothetical picture of a ganglionic receptor*

One possible additional receptor group would be that which interacts with the oxygen atom of choline phenyl ether, assuming Hey's hypothesis that this is important and should, for activity, carry a partial positive charge The difference between the activity of the optical isomers of nicotine indicates that in these compounds other factors must also be taken into account Possibly in the less active (+)-R-isomer the pyrrolidine ring is so placed that the affinity for the receptor is considerably reduced This suggests that there may be a limit to the size of the groups which can be tolerated at a particular position fairly close to the onium group The existence of anchoring groups some further distance away, as suggested by Gill and ing (1958), might account for the stereospecificity of substances such as *Trimetaphan* and the Tropine derivatives discussed on page 161 It is possible, therefore, to draw a picture of the receptor (Fig VI 6), but it must be emphasized that such a picture is entirely conjectural

Actions at Ganglia Other Than at Receptors on the Postganglionic Neurone

Although it has heen assumed that substances which stimulate or block transmission in autonomic ganglia do so by an action at the receptors on the postganglionic neurone, it is conceivable that they might also act at the pre-synaptic nerve terminals Riker and Szreniawski (1959) have shown that the intra-arterial injection of acetylcholine close to the superior cervical ganglion

of the cat causes the appearance of antidromic action potentials in the pre-ganglionic fibres as well as of action potentials in the postganglionic fibres Similar results were obtained with tetraethylammonium, and the anti-dromic potentials were reduced by the prior injection of Hexamethonium It was suggested that this shows that part of the actions of ganglion stimulant and ganglion blocking drugs may be at the presynaptic nerve-terminals, but it may be questioned whether such an action is important for most drugs which have been studied so far

Tetraethylammonium and compounds, such as _HC 3_ (page 138), are known, however, to interfere with the release of the transmitter at the neuromuscular junction, and a similar action in interfering with the release of the transmitter at ganglia might explain why recent estimates of the activity of tetraethylammonium (e g in Tables VI 7 and VI 17) make it appear much more active relative to substances such as Hexamethonium than earlier tests In recent work the drugs have been tested for their effects in reducing the sustained contracture of the nictitating in response to a con tinuous stream of impulses applied to the preganglionic nerve trunk In early work the preganglionic fibre was stimulated only at regular intervals, producing a contracture lasting for a relatively short time, and the ganglion left for several minutes to recover between stimuli Chou and Elio (1947), for instance, stimulated for 15 seconds every 3 minutes A substance which interfered with the release of the transmitter would appear much more active in the tests using continuous stimulation than in the tests using inter mittent stimulation

There may be additional ways of affecting conduction in ganglia Histamine has long been known to cause a release of adrenaline and _noradrenaline_ from the adrenal medulla (Burn and Dale, 1926) and Konzett (1952) Burn and Trendelenburg (1954) and Trandelenburg (1954) have presented evidence that histamine can stimulate sympathetic ganglia Gertner and Kohn (1959), however, have shown that, on the perfused superior cervical ganglion of the cat, histamine depressed conduction but only when given in relatively high doses As a blocking agent it was rather weaker than tetraethylammonium and, in doses which did not themselves produce much effect, it increased the block produced by tetraethylammonium or Hexamethonium and also that produced by substances such as tetramethylammonium or nicotine These results suggest, though they do not prove, that histamine acts at different receptors than those affected by tetraethylammonium and nicotine

Gaddum and Hameed (1954) suggested that in the guinea pig ileum there are two types of receptor, one sensitive to nicotine and the other to 5 hydroxy tryptamine, and Trendelenburg (1956) has suggested that this may also be true in the superior cervical ganglion of the cat When injected intra arterially close to the ganglion, 5 hydroxytryptamine produced a contracture of the nictitating membrane which was not blocked by Hexamethonium It appeared to be due definitely to an action on the ganglion because there was no con-tracture if the ganglion had been removed

It is difficult to see how far such receptors in the sympathetic ganglia may

he important pharmacologically, but it is not difficult to appreciate the importance of receptors in the ganglia of the intestine which are not cholinergic The picture of the autonomic connexions of the gut as being composed of sensory fibres leading to cholinergic parasympathetic ganglia with post-
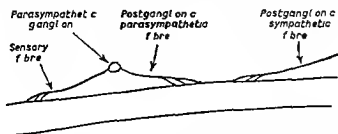
FIG VI 7 *Autonomic nervous connexions in the gut (diagrammatic)*

ganglionic parasympathetic fibres, and of postganglionic sympathetic fibres from the sympathetic chain (Fig VI 7) is almost certainly much too simple Ambache (1951) made use of *Botulinus* toxin, which has a selective affinity for cholinergic nerve endings, to produce a cholinergic blockage In these conditions nicotine produced relaxation of the intestine, an effect similar to
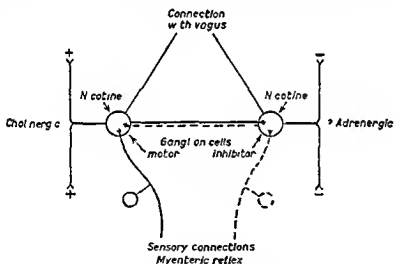
FIG VI 8

+ *susceptible to Botulinus toxin*
− *resistant to Botulinus toxin*

(*Ambache, 1951, Brit J Pharmacol, reproduced by permission*)

that produced by adrenaline This effect was antagonized by Hexamethonium and also by ephedrine (page 308) This suggests the existence of sympathetic ganglia in the intestine, and Ambache suggested that these and the parasympathetic ganglia might be interconnected in the way shown in Fig VI 8 Much still remains to be explained, the antagonism of the action of nicotine by ephedrine, for example, and the findings of Ellis and Rasmussen (1951)

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that nicotine produced effects, apparently by a nervous mechanism, on the intestine of dogs and rabbits even in the presence of atropine, antiadrenaline, and antihistamine drugs. The complexity of the connexions in the intestine may well account for the difficulties experienced in producing drugs which are really effective in the treatment of conditions such as those leading to the formation of ulcers.

Conclusion

Many compounds are known which affect the transmission of nerve impulses across ganglia, some of these are of practical value and something is known about how they act. Nevertheless, it cannot be pretended that we really understand very much about what is going on, particularly in parasympathetic ganglia.

VII

Actions at Cholinergic Synapses
III Postganglionic Cholinergic Receptors

Transmission at Postganglionic Cholinergic Nerve-endings

The receptors discussed in this chapter are those on muscles or organs innervated by parasympathetic nerves and by cholinergic sympathetic fibres, such as those supplying certain sweat glands, and the acetylcholine receptors on blood vessels, which may be innervated by cholinergic sympathetic fibres or may have no nervous connexions at all Anatomically the arrange ment of these nerve endings and muscle fibres is quite different from the arrangement at the neuromuscular junction or in ganglia There is no organized end plate, and the use even of the term 'synapse' may convey an impression of a greater degree of organization than is so far known to exist Part of the apparent complexity can be ascribed to the difficulty of studying such structures experimentally and part to the great variety of cells on which these receptors are located The most important receptors of this type are those in the heart, in the salivary glands in the stomach and intestine, in certain sphincters such as that of the bladder, in the circular muscle of the iris and in the ciliary body, in certain blood vessels and in the sweat glands

Transmission at these sites is strikingly different from transmission at the neuromuscular junction and in ganglia even though acetylcholine is the transmitter at both types of site The effects are much less precise events may take seconds, whereas comparable events at the neuromuscular junction require only milliseconds

One particularly striking difference is that, unlike voluntary muscle where the response to stimulation is all or none, the tension developed by smooth muscle fibres appears to be continuously variable up to the maximum of

185

which the fibres are capable The fibres are actually composed of many units, each of which may respond in an all or-none fashion, but the whole object functions as a syncytium (i e as a single structure) The response, therefore, appears to be continuously variable, though it may really be composed of a number of quanta

The electrical events occurring at smooth muscle have been studied by Bozler (review, 1948), using external electrodes, and more recently by Bülbring (review, 1957) The resting potential of most types of smooth muscle, recorded by direct measurement with an intra-cellular electrode, is lower than that of voluntary muscle, being somewhere around 50 mV, but the potential varies greatly with the tension of the muscle

In experiments with the *taenia coli* of the guinea-pig, a thin longitudinal muscle located in the colon and easily dissected out, Bülbring (1954) ob-

FIG VII 1   *Electrical and mechanical records from the taenia coli of the guinea pig a micro-electrode was inserted inside a single muscle cell, and the record is of the potential difference between this and an electrode placed outside the cell superimposed on the resting potential there is a series of spikes indicating depolarization The steady line shows the tension in the muscle and a contraction, i e an increase in tension, brought about in this experiment by increasing the K^+ concentration, is associated with an increase in the frequency of the spikes and a slow depolarization of the membrane of the muscle cell (Holman, 1958)*

(From J Physiol, 141, 464, Fig 5 (1958) Reproduced by permission)

served a considerable amount of spontaneous activity There were slow changes in the tension of the muscle and in the polarization of the cell membrane Superimposed on these gradual fluctuations were a series of spikes, rapid depolarizations occurring normally about once a second, but much more frequently during bursts of activity (Fig VII 1, Holman, 1958) Bülbring (1954) found that acetylcholine (1.5×10^{-8} M) caused a relatively slow depolarization of the membrane and an increase in the frequency of the spikes A similar effect was observed with histamine (10^{-6} M), but adrenaline (1.5×10^{-6} M) increased the polarization of the membrane and greatly reduced the frequency of the spikes, as well as reducing the tension in the muscle The frequency of the spikes increased with increasing tension in the muscle, and vice versa, but was not reduced by Hexamethonium (3×10^{-4}– 10^{-3} M) or by cocaine (2×10^{-6} M, a concentration which blocks the peristaltic reflex) It was reduced by a high concentration of atropine (around 10^{-4} M), lower concentrations did not alter the frequency, but did alter the response of the muscle to stretching (Bülbring, 1955)

By analogy with the neuromuscular junction it might be supposed that acetylcholine is released in quanta from the nerve-endings and that the spikes are comparable with miniature end plate potentials, but the size of the spikes (with suitable electrodes these are seen to be greater than the resting potential) and the failure of Hexamethonium or cocaine to reduce their frequency indicate that this is not so moreover, this particular tissue actually contains no nerve-cells The spikes arise as a normal consequence of activity in the muscle-cell and the action of acetylcholine must be upon the mechanism giving rise to the spikes, not, as at the end plate in the neuromuscular junction, by a depolarization of the membrane itself There is thus some parallel between the smooth muscle of the *taenia coli* and cardiac muscle Although cardiac muscle was included in the list of tissues containing postganglionic cholinergic receptors, it may seem unjustified to compare such spontaneously active tissue with the apparently passive or intermittently active smooth muscle of the gut, etc The action of acetylcholine on the heart, for instance, is both on the rate of beating, i e on the pace-maker, and on the force of contraction, whereas the action on a piece of intestine is often seen, at least *in vitro*, simply as a contraction of the muscle The action of acetylcholine on the process in the pace maker which gives rise to action potentials and beating of the heart could well be comparable with the action on the process in the *taenia coli* which gives rise to spikes

The action potentials and pace maker-potentials of heart muscle appear, like the action potentials in nerve fibres, to be attributable to the movements of ions through the cell membrane, and Hutter and Noble (1961) and Noble (1961) have shown that it is possible to modify the sort of equation shown on page 50 to account satisfactorily for the shape of the action- and pace-maker-potentials It seems probable that the spikes observed with the *taenia coli* may similarly be attributed to movements of ions (Born and Bulbring, 1956) and that the actions of substances like acetylcholine, histamine, and adrenaline may be linked with changes in the permeability of the membrane to K+ and other ions The effects of acetylcholine on the resting potential of heart muscle-fibres, however, are different from those on voluntary or smooth muscle fibres such as the *taenia coli* Instead of becoming depolarized the fibres become more polarized (review, Hutter, 1957), an effect thought to be due to an increase in the permeability to K+.

Although, therefore, there are certain similarities in the behaviour of all types of muscle and the effects of transmitters may be essentially the same for each, a modification of the permeability of the muscle membrane to ions, smooth muscle must be distinguished from voluntary muscle because of its characteristically slower response, and heart muscle must be distinguished because acetylcholine increases polarization instead of decreasing it Yet in spite of the fact that the effects of acetylcholine on the polarization of smooth muscle are exactly the opposite of its effects on the polarization of cardiac muscle, it will be seen that the relationships between chemical structure and pharmacological activity are remarkably similar at both sites, suggesting that the receptors are similar in structure

Antagonism of the Action of Acetylcholine at Postganglionic Cholinergic Receptors

The best-known antagonist of acetylcholine at these sites is atropine, and this appears to act by competition with acetylcholine Clark (1926) observed the concentrations of acetylcholine which, in the presence of various concentrations of atropine, reduced the rate of beating of the frog heart to half He found that the drug-ratio (page 43) was constant except at very high and very low concentrations, and in 1937 showed that the results fitted Gaddum's equation $A/a = 1 + BK_B$ (page 12) for competitive antagonism Clark used the results for acetylcholine alone (a) and in the presence (A) of one particular concentration of atropine (B) to calculate K_B, and then computed the other concentrations of acetylcholine which should produce the same effect in the presence of other concentrations of atropine The calculated and observed concentrations agreed remarkably well (Table VII 1)

TABLE VII I

Antagonism of Acetylcholine by Atropine in the Frog Heart

Atropine concentration	Acetylcholine concentration reducing the rate of beating to half	
	Observed	Calculated
0	1	t
10^{-8} M	$t 6 \times 10^{-8}$ M	$1 3 \times 10^{-8}$ M
10^{-7}	$3 6 \times 10^{-6}$	$4 0 \times 10^{-6}$
10^{-6}	$2 5 \times 10^{-5}$	$3 1 \times 10^{-5}$
10^{-5}	$3 0 \times 10^{-4}$	$3 0 \times 10^{-4*}$
10^{-4}	$3 5 \times 10^{-3}$	$3 0 \times 10^{-3}$
10^{-3}	$4 7 \times 10^{-2}$	$3 0 \times 10^{-2}$

* This result was used for calculating K_B

Clark (1926, 1937)

Further evidence for competition was provided by Schild (1960) who observed that, with the guinea pig ileum, the graph of the logarithm of the value, *dose-ratio minus 1*, against the logarithm of the concentration of atropine was a straight line with a slope of unity The value of the logarithm of the concentration of atropine for which the logarithm of the *dose ratio minus 1* was zero should be $-\log K_B$, and the association constant K_B for atropine and the receptors in the guinea pig ileum was found to be approxi mately $10^{9 \cdot 1}$ This is rather higher than estimates obtained from measuring pA_2, $10^{8 \cdot 8}$ (Schild, 1947)

Marshall (1955) used the difference between pA_2 and pA_{10} as an indication of competition (page 44) and obtained results which suggested that the antagonism of atropine with acetylcholine was non competitive, although the action of (−) hyoscyamine (atropine is the racemate, see page 214) was apparently competitive This method is probably not as reliable as the method

used by Schild The errors attached to the estimates of pA may be quite considerable compared with the expected difference between the two values (see page 44) A better procedure is to plot the *dose ratio minus 1* against the concentration of antagonist, if the antagonism is competitive the graph should be a straight line passing through the origin (Fig II 11)

Although the actions of some antagonists of the muscarine like actions of acetylcholine have been tested to see if they are competitive (usually on the guinea-pig ileum for reasons of convenience), the vast majority of antagonists have not been tested in this way

Desensitization and Tachyphylaxis

After large doses of acetylcholine, or of other agonists such as histamine, most smooth muscle-fibres become insensitive for a considerable time This 'tachyphylaxis' might be due to desensitization produced in a way similar to that of substances like Decamethonium at the neuromuscular junction, or another explanation could be that, as proposed by Paton (1961), it is the rate of combination of drug with receptor which is important for obtaining a response, and after saturation of the receptors with the drug this rate can only be low Paton himself rejects this explanation for tachyphylaxis, because the insensitivity often extends to other agonists which act on different receptors, and points out that the insensitivity could be due to metabolic exhaustion of the tissue, for instance, to depletion of K^+ (Cantoni and Eastman, 1946, Rand, 1957, Bulbring and Burnstock, 1960) Whatever may be the explanation, and although the phenomenon is easy to demonstrate, there do not appear to be drugs which exclusively block the receptors by producing tachyphylaxis in a way analogous to the desensitization produced by Decamethonium at the neuromuscular junction An explanation for this may be that because the response of smooth muscle is not all or none, there is a wide range between the concentrations producing threshold and maximal effects Consequently it is impossible to overlook or avoid the initial stimulation which would be characteristic of antagonists of this type

Uses of Substances Which Act at Postganglionic Cholinergic Receptors

Substances which act like acetylcholine at postganglionic cholinergic sites (sites of the muscarine like actions of acetylcholine), find a very limited use in circumstances where it is desired to stimulate the parasympathetic, possibly because it is not functioning satisfactorily Such drugs are used, for example, to overcome retention of urine when the wall of the bladder fails to contract or the sphincter fails to relax, also in the condition known as post operative paralytic ileus, in which, for some reason, the gut is distended with gas which is expelled by the contraction induced by the acetylcholine like drug Another use is in glaucoma, a disease in which the pressure inside the eye is too high and stimulation of the cholinergic receptors in the ciliary body may relieve the pressure because contraction of the ciliary body improves drainage through the nearby canal of Schlemm, it also causes the lens to become more spherical and focused for near vision In practice, substances which have the

muscarine like properties of acetylcholine do not act long enough to be of lasting value and anticholinesterases are normally used instead (page 243)

Antagonists of acetylcholine have a variety of uses Atropine or hyoscine (see below) is routinely used pre-operatively to stop secretions which may be stimulated to excess during anaesthesia and cause the patient to drown (ether is a particularly effective stimulant of bronchial secretion), and it will also protect the heart from excessive vagal stimulation which might arise during the operation and stop it How far this is genuinely likely to occur is difficult to assess, because an atropine-like drug is invariably used to stop secretions

Atropine like compounds are also useful as antidotes for poisoning with an anticholinesterase The immediate toxic effects due to the accumulation of acetylcholine are at the postganglionic cholinergic receptors in the heart, intestine, salivary glands, and eyes and should all be blocked by atropine

In order to examine the eye it is often necessary to dilate the pupil, and to rest the eye it may be necessary to paralyse the accommodation, for both these purposes substances acting like atropine are useful, although usually atropine itself is not used because its effects last too long

One of the greatest potential uses of atropine like compounds is to reduce gastric secretion and gastric and intestinal motility in patients with ulcers This has been mentioned in connexion with ganglion blocking agents, and the large number of compounds which have been tested and marketed is a fairly good indication that no particular compound is outstandingly effective

Testing of Drugs on Postganglionic Cholinergic Receptors

Preporations

In studying the actions of drugs on postganglionic cholinergic receptors it is impossible, except when the receptors are part of the sympathetic system to separate them from the ganglia which innervate them In Loewi's experiments with the perfused hearts (page 80) for instance it is not impossible that the acetylcholine liberated from the first heart originates from the parasympathetic ganglia and acts on receptors in the parasympathetic ganglia of the second heart Perry and Talesnik (1953) have, in fact, shown that the slowing of the heart produced by small doses of acetylcholine is reduced or blocked by Hexamethonium and consequently is at least partly due to stimulation of the intracardiac ganglion cells of the vagus rather than to an action of the postganglionic cholinergic receptors As a rule, however, the postganglionic cholinergic receptors are more sensitive to acetylcholine than the receptors in ganglia This is seen in the actions of acetylcholine on the blood pressure In the cat, a small dose (about 10 nMoles, 1 nMole = 10^{-9} g Mol) produces dilatation of certain blood vessels and a consequent fall in blood pressure larger doses (about 250 nM) also slow the heart It is only with very much larger doses (about 25 μM) that there is a rise in blood pressure because of stimulation of the sympathetic ganglia and, possibly, of the adrenal medulla

The ability of drugs to lower the blood pressure is accordingly often used for estimating muscarine-like activity and should be abolished by atropine

This experiment can be performed in an anaesthetized animal, usually a cat or a rat, or in a 'spinal' animal in which the brain has been destroyed. In the latter, respiration must be maintained by a pump because the respiratory centres in the medulla will no longer be functioning. The blood pressure can be recorded directly by a mercury manometer connected to the carotid artery and drugs are given by injection usually into the femoral vein. It is also possible to record the blood-pressure in conscious animals by suitable pressure-gauges. The chief disadvantage of this type of experiment is that the drug is exposed to the action of destroying enzymes all over the body, the cholinesterase of plasma often being particularly important. Although it may yield information about the possible clinical effects of a drug it may not be a reliable indication of its fundamental activity.

Drugs can be tested for their effects on the rate and force of the beating of an isolated heart. In the preparation described by Langendorff (1895, Gunn, 1913), a cannula is inserted into the aorta and the perfusion is backwards, through the coronary circulation, the pressure of the perfusion fluid closing the valve in the left ventricle. In the preparation used by Strauh (1910), the cannula is passed through the valve and into the left ventricle, but even in these conditions the chambers of the heart are not filling and emptying normally. More normal conditions are achieved in the heart-lung preparations of Jerusalem and Starling (1910), Patterson and Starling (1914) and of Evans, Grande, and Hsu (1934), but these are much more complicated, and the drug is exposed to destruction, e.g. by enzymes in the blood. Useful information may be obtained with a much simpler preparation, obtained by dissecting out only the auricles of a guinea pig or rabbit heart and mounting these in an organ bath. These should continue to beat regularly, and the effects of drugs on the rate and force of the beat can be recorded by fixing one end of the preparation and attaching the other to a light lever.

The actions of drugs on the postganglionic cholinergic receptors of the gut can readily be studied on the isolated guinea pig ileum (page 144) and in whole, anaesthetized, animals by attaching threads to a portion of the gut and recording the movement with a suitable system of pulleys and levers.

Effects on salivation can be studied by cannulating the salivary duct, usually of a cat, and collecting the effluent, using some kind of drop-recorder to measure the flow (Bulbring and Dawes, 1945, Brown and Quinton, 1957). Salivation can be induced either by stimulation of the nerve supply, the *chorda tympani,* or by injecting a muscarine-like compound. Gastric secretion is more difficult to study, but it is possible to measure both the volume and the acidity of gastric juice produced in response to administration of a muscarine-like drug (Ghosh, 1958, Ghosh and Schild, 1958).

The actions of drugs may also be studied on the urinary bladder. Lourie (1952) has described a method of mounting this which is rather similar to the Trendelenburg preparation (page 144). The ureters, conveying the urine from the kidney to the bladder, are tied off and the exit (the urethra) is cannulated. The bladder (upside down) is filled with physiological saline and immersed

in an organ bath There is a slight bead of pressure between the fluid inside and outside the bladder, the greater pressure inside keeping the bladder distended Drugs are added to the outer bath and their effects on this pressure-bead can be recorded by means of a suitable piston-recorder

The actions of drugs on the pupil of the eye can readily be observed in small animals such as mice. The pupil contains two muscles, the radial, which is sympathetic, and the circular, which is parasympathetic Contraction of the radial muscle dilates the pupil, whereas contraction of the circular muscle, operating in the same way as pulling the strings of a sponge bag, constricts the pupil A drug which antagonizes the actions of acetylcholine should block the action of the parasympathetic and lead to dilatation of the pupil (mydriasis) Pulewka (1932) and Ing, Dawes, and Wajda (1945) have used the size of the pupil of the mouse to measure this effect The size of the pupil (measured with a binocular microscope) should depend upon the degree of block of the parasympathetic cholinergic receptors, and also upon other factors including the sympathetic tone Although it is not possible to measure the degree of antagonism of acetylcholine as such, it is possible to compare the effects produced by different antagonists by comparing the mean size of the pupils of groups of mice at n standard time after the injection of the drugs A graph of pupil size against log dose is linear over part of the range, and these graphs can be compared for different drugs

Singh Grewal (1951) has modified the test so that it can be used for substances which act like acetylcholine Mice are given a standard dose of atropine together with different doses of the acetylcholine-like compounds Over a certain range the size of the pupil should then be inversely related to the logarithm of the dose of the drug It might be possible to modify this test still further and, by a judicious selection of the doses of agonist and antagonist to offset the dilatation by the constriction The test could then be used to analyse the antagonism to see if it were competitive, but this does not appear to have been tried The method is probably too tedious and complicated to be convenient

The actions of drugs on the blood-vessels can be studied on such preparations as the perfused rabbit's ear or the perfused dog's hind limb or rat's hindquarters The perfusion is made through the arterial supply and the effluent collected (on a tray or over a funnel, there is no need to cannulate the veins) The effects of vasodilator drugs on the rate of flow can easily be studied, but vasoconstrictor drugs are more difficult to work with because too large a dose may stop the flow altogether and the drug cannot be washed out In all experiments involving atropine (and possibly other esters) and rabbits the results may be greatly complicated by the presence in some animals, but not others, of an enzyme which hydrolyses atropine (Glick, 1940)

Agonist Activity

With all the preparations listed above, except the perfused blood-vessels, it may be necessary to show that the drugs really affect the postganglionic

cholinergic receptors and not the receptors in parasympathetic ganglia If the effects are not modified by ganglion-blocking agents the agonist activity of one drug relative to another can be determined by comparing the concentrations which produce identical effects and, if the dose-response curves are parallel, expressed as an equipotent molar ratio In tests using the blood-pressure, heart, intestine, and bladder, the time course of the action should be clear In tests using salivation, gastric flow and perfused blood vessels results may be complicated by a time lag before the response In experiments with the eye the effects are usually measured after a standard time interval and give no impression of the time-course of the action of the drug In this test matters are further complicated because the drugs are often given by intra-peritoneal injection, and the results may depend upon the relative rates of absorption It is with the blood pressure, heart, intestine, and bladder, therefore, that it is easiest to see whether drugs are capable of producing identical effects and, accordingly, whether an estimate of the equipotent molar ratio has any meaning

Although measurements of the concentrations of agonists which produce half the maximal response (pD is the logarithm of the reciprocal of this concentration, page 8) have sometimes been made, it is possible only in a few instances to measure agonist activity properly in terms of association constants and efficacy (page 9) More fundamental information about the actions of drugs might also be obtained by studying their effects on electrical events in the tissues rather than the gross physiological response This type of experiment, however, will have to wait until more is known about the significance of those electrical events which have been observed so far

Antagonist Activity

In the tests with the blood pressure, ileum, bladder, and blood vessels, antagonists of the actions of acetylcholine will have little effect on their own On the heart, salivation, gastric juice, and the eye, antagonists will produce an observable effect – increased pulse rate, decreased secretion, dilatation of the pupil – because there is normally a balance between the sympathetic and the parasympathetic and this has been disturbed Even with the latter group of preparations, however, it is more convenient to study the effect of an antagonist on the response to an agonist, rather than to use the response to the antagonist alone as an effect on which comparisons are based The normal salivary flow, for instance, is relatively low, and a dose of atropine will usually either abolish it completely or fail to produce a detectable effect The comparison of atropine like drugs under these conditions could, accordingly, only be made at one dose level If an agonist is used to stimulate the flow, however, quite a range of concentrations of antagonists could be studied and the drugs compared more adequately

In experiments with isolated intestine the effect of an antagonist on the spontaneous activity may be observed, but if a stimulant is used (e g acetyl choline or Carbachol) a wider range of concentrations of antagonist can be studied With guinea pig intestine spontaneous activity is very slight, which

is an advantage if accurate assays are to be made On this preparation an antagonist must be tested for its effects in antagonising an agonist or the effects of transmural electrical stimulation as described by Paton (1955)

The measurement of antagonist activity in terms of the affinity constant could, in fact, be undertaken with almost all the preparations listed above The evaluation of K_B by determining the dose ratio over a considerable range of concentrations of antagonist is more reliable than estimates only of pA2, but these are often quite reasonably consistent and informative Complications seem chiefly to arise when the development of equilibrium with the antagonist is slow This is particularly noticeable with atropine itself and probably accounts for the differences between K_B determined graphically and K_B as determined from pA2 (page 188) Schild (1947), for instance, observed that after 2 minutes contact with the guinea pig ileum the pA2 for atropine was 8 27, whereas after 14 minutes it was 8 61 With other substances equilibrium appeared to be reached much more rapidly, the corresponding figures for the drug Pethidine were 5 79 and 5 84 respectively With quaternary compounds, where there should be no complications arising from the passage of drug into the cells, the achievement of equilibrium should be particularly rapid and estimates of K_B from pA2 correspondingly more reliable

In spite of the ease with which K_B could be determined, the activities of many compounds have often been expressed only in terms of that of another antagonist (usually atropine) If these are expressed as an equipotent molar ratio the value for K_B can easily be deduced from that of atropine

AGONISTS

Activity of Simple Onium Salts

Compared with their effects at the sites of the nicotine-like actions of acetyl choline, simple onium salts are only feeble at the sites of muscarine-like activity At the receptors in the heart and gut the equipotent molar ratio of tetramethylammonium relative to acetylcholine is of the order 500 to 1,000 (Clark and Raventos 1937) Higher tetra alkylammonium salts, such as tetraethylammonium and tetrapropylammonium, were only antagonists (Külz, 1923) Agonist activity in alkyltrimethylammonium salts, however, depends on the length of the alkyl group and is maximal in n pentryltri methylammonium (Table VII 2) The chain length appears to affect both affinity and efficacy (Stephenson, 1956), affinity increasing almost in a geometrical progression The value of K for n heptyltrimethylammonium was 41×10^3, for the n octyl compound, 63×10^3, for n nonyl 110×10^3, and for n-decyl 190×10^3 The effects on efficacy are entirely different there being a decline from tetramethylammonium to ethyltrimethylammonium and a maximum at n butyltrimethylammonium and n pentryltrimethylammonium If the equipotent molar ratio for tetramethylammonium relative to acetylcholine is taken as 1,000, the value for n pentyltrimethylammonium relative to acetylcholine should be of the order of 10 This appears to be about right, in a direct comparison, Ing, Kordik, and Tudor Williams (1952) obtained the

ratios 66 on the cat blood pressure, 44 on rabbit auricles, 8 on the guinea pig ileum, and 325 on the frog heart Welsh and Taub (1950, 1951) obtained the value 70 using the heart of the clam, *Venus mercenaria*, but the receptors in this tissue appear to resemble those at the sites of the nicotine like actions of acetylcholine, being insensitive to atropine Although there is some variation in the estimates shown in Table VII.2, particularly with certain preparations

TABLE VII.2

Activity of Alkyltrimethylammonium Salts Equipotent Molar Ratios Relative to Tetramethylammonium

$\overset{+}{\text{RNMe}_3}$

Preparation	Me	Et	*n*-Pr	*n* Bu	*n* Pent	*n* Hex	HOCH$_2$CH$_2$ (Choline)
Frog heart (K)	1	>100	10–30	0 10–0 15	0 50–1 0	—	—
(R)	1	2	1	0 020	0 010	>1	—
Dog blood pressure (A and K)	1	1	0 20	0 02	0 02	0 10	—
Tortoise auricle (L)	1	4 2	7 2	0 13	0 071	—	3 6
Venus heart (W and T)	1	0 77	0 017	0 012	0 0023	0 017	—
Rabbit intestine (A and K)	1	2	1	0 10	0 05	0 10	—
(L)	1		0 48	0 16	0 081	—	2 8
Raj jejunum (VR and A)	1	1 6	0 50	0 050	0 032	0 079	5 0
Guinea pig ileum (S)	1	1 2	4 5	0 031	0 011	0 048	—
Affinity constant (K)	0 24 × 10^3	0 63	1 6	3 8	8 5	19 0	—
Efficacy (e)	94	31	4 3	200	200	21	—

K = Kulz (1923) R = Raventos (1937) A and K = Alles and Knoefel (1939) L = Lands (1951) W and T = Welsh and Taub (1950 1951) VR and A = Van Rossum and Ariens (1959) S = Stephenson (1956)

such as the clam heart, it is clear that the chain length has a profound effect on activity and is optimal in the *n* pentyl group

Hunt and Renshaw (1925) tested the phosphorus arsenic antimony, and sulphur analogues of tetramethylammonium on the blood pressure of the anaesthetized cat and found that these were all less active than the quaternary ammonium compound The arsenic and antimony analogues were about equiactive and less active than the phosphonium compound which was less active than trimethylsulphonium

Phenyltrimethylammonium and benzyltrimethylammonium were slightly more active than tetramethylammonium in causing a fall in the blood pressure of the cat (Hunt, 1926), but β phenylethyltrimethylammonium was virtually inactive (Hunt and Renshaw, 1933)

Effects of Altering the Onium Group in Acetylcholine

The effects of altering the onium group in acetylcholine itself have been discussed by Ing (1949), Ing, Kordik, and Tudor Williams (1952), and Scott (1962) Some of the results are summarized in Table VII 3 Activity appears to

TABLE VII 3

Activity of Acetoxyethyl Onium Salts Equipotent Molar Ratios Relative to Acetylcholine

CH₃COOCH CH₂−	Cat blood pressure	Intestine	Frog heart
$\overset{+}{N}Me_3$	1	1 (Rabbit)	1
$\overset{+}{N}Me_2H$	50	40	50
$\overset{+}{N}MeH_2$	500	1 000	500
$\overset{+}{N}H_3$	2 000	20 000	40 000

Stehle Melville, and Oldham (1936)

NMe_2Et	3	2 5 (Guinea pig)	2
$\overset{+}{N}MeEt_2$	400	700	1,500
$\overset{+}{N}Et_3$	2 000	1 700	10 000*
Bis A	150	100	75
Tris A	20 000	20 000	12,500

Bis A = $(CH_3COOCH_2CH_2)_2\overset{+}{N}Me_2$ Tris A = $(CH_3COOCH_2CH_2)_3\overset{+}{N}Me$

Holton and Ing (1949)

$\overset{+}{P}Me_3$	13	12 (Rabbit)	12
$\overset{+}{A}sMe_3$	66	90	83

Welch and Roepke (1935)

$\overset{+}{S}Me_2$	50	30 (Guinea pig)	96

Ing Kordik and Tudor Williams (1952)

Size of quaternary atom		
N	$d = 1 47$ Å	$d = 2 4$
P	1 87	3 05
S	1 82	—
As	1 98	3 23

* Reduces effect of acetylcholine

he dependent upon the size of the central onium atom, although the sulphonium analogue is less active than the phosphonium analogue Ing (1949) pointed out that although activity is reduced by the replacement of one

metyl group by ethyl, the replacement of a second methyl group produces a much greater decrease he suggested that this could be explained by supposing that the onium group is adsorbed at a plane surface or hemispherical cavity (page 118) Replacement of one methyl group would still leave two attached to the onium atom so the molecule could still be adsorbed, although only in one conformation With replacement of the second methyl group by ethyl, the ability to fit should be destroyed completely The work of Scott (1962), however, has shown that the affinity is increased when methyl groups are replaced by ethyl in the onium group, being greater for the ethyldimethyl and methyldiethyl compounds than for the trimethyl or triethyl the decrease in activity brought about by replacing trimethylammonium by ethyldimethyl ammonium can be ascribed to a drastic reduction in efficacy It seems likely, therefore, that it is necessary to compromise and suppose that it is the size of the onium group, and the need for at least two methyl groups attached to it which is necessary for efficacy It would, of course, be helpful if more was known about the mode of action of acetylcholine so that the meaning of 'efficacy' could be understood

Effects of Altering the Acyl Group in Acetylcholine

Replacement of the acetyl group in acetylcholine by other acyl groups invariably leads to a decline in muscarine like properties The activity of some of the simpler aliphatic esters of choline is shown in Table VII 4

TABLE VII 4
Activity of Esters of Choline Equipotent Molar Ratios Relative to Acetylcholine

Compound	Blood pressure (fall)			Intestine			
	Cat (S)	(W)	Rabbit (C and G)	Rabbit (C and G)	Guinea pig		
					(A P, and S)	(Le H)	(W)
Choline	—	—	20 000	133	1 000	1 000	—
Formyl	—	—	—	—	20	10	—
Carbamoyl	—	—	6 6	1 2	—	—	—
Pyruvyl	—	—	10	7	—	—	—
Acetyl	1	1	1	1	1	1	1
n Propionyl	5	37	28	33	10	3	19
n-Butyryl	125	2 900	inactive	400	—	25	510
n Valeryl	—	—	inactive	500	—	66	—

*A P and S = Abderhalden Paffrath and Sickel (1925) Wertheimer and Paffrath (1925)
C and G = Clang and Gaddum (1933) Le H = Le Heux (1921) S = Simonart (1932)
W = Wurzel (1959)*

Homologues higher than butyrylcholine have been shown to be antagonists of acetylcholine (Schneider and Timms 1957) Dimethylacryloylcholine and similar compounds have only feeble muscarine-like activity (Holmstedt and

Whittaker, 1958), and this is also true of murexine and compounds related to
it (Erspamer and Glässer, 1958). Aromatic esters of choline, likewise, are
feeble. The equipotent molar ratio for benzoyl choline relative to acetyl-
choline on rabbit intestine, for instance, is of the order of 1,000 (Ackasu,
Sinha, and West, 1952; Wurzel, 1959), and phenylacetylcholine and β-phenyl-
propionylcholine were considered by Hunt and Taveau (1911) to be devoid
of muscarine like activity

The most active esters appear to be those of carbamic acid (*Carbachol*,
VII 1, Kreitmair, 1932) and of formic acid. Although the values for the

$$H_2N-C\underset{O-CH_2CH_2\overset{+}{N}Me_3}{\overset{O}{\diagup}}$$

Carbachol, VII 1

equipotent molar ratio will be considerably influenced by the relative stability
of the compounds, and *Carbachol* is much more slowly hydrolysed than
acetylcholine, it is clear that these compounds have appreciable *muscarine*-
like activity, although they are not as active as acetylcholine

Effects of Altering the Choline Part of the Molecule

Either shortening or lengthening the chain in acetylcholine reduces muscarine-
like activity (Hunt and Taveau, 1911, Hunt and Renshaw, 1925, 1934).
Acetyl *nor*-choline (acetylformocholine, acetoxymethyltrimethylammonium,
VII 2) is not very stable, but its feeble activity cannot be ascribed only to this

$$CH_3-C\underset{O-CH_2-\overset{+}{N}Me_3}{\overset{O}{\diagup}}$$

VII 2

The addition of a branched methyl group to the chain markedly affects
activity. Acetyl α methylcholine has nicotine like activity but little muscarine-
like activity, whereas acetyl β methylcholine (*Mecholyl*) has muscarine like
activity but little nicotine like activity (Simonart, 1932). Acetyl β methyl
choline was resolved by Major and Cline (1935) and it was found that the
(+)-isomer was much more active than the (—)-isomer even though the
(+)-isomer is more rapidly destroyed by cholinesterase (Glick 1938). The
activities of the compounds are shown in Table VII 5. Ellenbroek and Van
Rossum (1960) have shown that (+) acetyl β methylcholine has the S-con-
figuration (VII 3) as might be expected by analogy with muscarine (page

$$CH_3-C\underset{O}{\overset{O}{\diagup}}\underset{H}{\overset{CH_2-\overset{+}{N}Me_3}{\diagdown}}$$

(+)-S-*Mecholyl*, VII 3

211) Beckett, Clitherow, and Harper (1960) have shown that (+)-β-methyl-
choline has the S-configuration, whereas (+)-α methylcholine has the R con-
figuration. As acetylation does not alter the sign of rotation of the β-methyl

TABLE VII 5

Activity of Isomers of Acetyl α- and β-Methyl Cholines Equipotent Molar Ratios Relative to Acetylcholine

	Frog heart	Rat intestine	Guinea pig ileum	Cat blood-pressure
(+)-CH$_3$COOCHMeCH$_2$$\overset{+}{\text{N}}Me_3$	6 3	1 3	1 0	0 75
(±)-CH$_3$COOCHMeCH$_2$$\overset{+}{\text{N}}$M $_3$	13	2 5	1 6	0 97
(−)-CH$_3$COOCHMeCH$_2$$\overset{+}{\text{N}}Me_3$	1,300	400	240	200
(+)-CH$_3$COOCH$_2$CHMeN$\overset{+}{\text{N}}$Me$_3$	—	—	28	13
(±)-CH$_3$COOCH$_2$CHMeN$\overset{+}{\text{N}}$Me$_3$	—	—	49	35
(−)-CH$_3$COOCH$_2$CHMeN$\overset{+}{\text{N}}$Me$_3$	—	—	230	140

Ellenbroek and Van Rossum (1960); Beckett, Harper, Clitherow, and Lesser (1961)

derivative, it seems likely that (+)-acetyl-α methylcholine should be R (VII 4)

(+)-R acetyl α methylcholine, VII 4

The thio-compounds, acetylthiocholine and acetyl-β-methylthiocholine are predominantly nicotine-like compounds with feeble muscarine like activity (Wurzel, 1959; Renshaw, Dreisbach, Ziff, and Green, 1938) Ethers related to acetylcholine have been studied by Dale (1914) and by Simonart (1934) and more recently by Ing, Kordik, and Tudor Williams (1952) The relative activities of these compounds and of analogous ketones are shown in Table VII 6 The ketones have only feeble muscarine-like activity and of the ethers, even the most active, the ethyl ether of choline, is not much stronger than *n*-pentyltrimethylammonium Aromatic ethers of choline, like the aromatic esters, have negligible muscarine-like properties

Some β-substituted piperidine derivatives, in which the choline portion forms part of a 6-membered ring, have been studied by Lands (1951) and are only feeble The most potent, the acetylester of N-methylpiperidine-3-carbinol (VII 5) was only as active as tetramethylammonium when tested on

VII 5

rabbit intestine and on the isolated tortoise auricle Activity was decreased by quaternization, the corresponding metho-salt being less than half as active, as was also the metho-salt of the methyl ester of piperidine-3-carboxylic acid

TABLE VII 6

Activity of Ethers and Ketones Related to Acetylcholine Equipotent Molar
Ratios Relative to Acetylcholine

$$\overset{+}{R}NMe_3$$

	Cat blood-pressure	Rabbit auricles	Guinea pig ileum	Frog heart
$CH_3(CH_2)_2OCH_2-$	49	39	61	392
$CH_3CH_2O(CH_2)_2-$	10	22	10	22
$CH_3O(CH_2)_3-$	39	44	98	294
$CH_3(CH_2)_4-$	66	44	8	325
$CH_3(CH_2)_2COCH_2-$	84	1,700	330	5,000
$CH_3CH_2CO(CH_2)_2-$	Only pressor	250	670	1,700
$CH_3CO(CH_2)_3-$	Only pressor	50	80	416
$CH_3COCH_2CH_2-$	150	70	167	230
	10–30	16	12	126
	1–3	1 2	0 34	15

Ing, Kordik, and Tudor Williams (1952)

The compound methyl-(β-trimethylammonium) propionate (VII 6, Schueler and Keasling, 1951) is isomeric with acetylcholine, differing from it only in that the ester group is reversed It is not hydrolysed by cholinesterases, and

VII 6

consequently estimates of its activity in whole animals are not very informative, but it appears, nevertheless, to have appreciable muscarine-like activity It was found to be as active as acetylcholine on the (isolated) guinea-pig ileum The methyl ester of γ-crotonic betaine (VII 7, Burgen and Hobbiger,

VII 7

1949) also has appreciable muscarine-like activity (in addition to nicotine-like activity), the equipotent molar ratio relative to acetylcholine was 2 on the guinea-pig ileum, 10 on the frog heart, and 12 on the cat's blood pressure

Other unsaturated analogues have been studied by Jacob, Marszak, Bardisa, Marszak-Fleury, and Eptsztein (1952), and appreciable activity has been found in a number of compounds in which the choline residue has been replaced by a longer chain containing an acetylenic bond (Table VII 7)

Muscarine-like activity on the cat heart has also been observed with unsaturated bis-onium salts which are analogues of Pentamethonium and

TABLE VII 7

*Activity of Analogues of Acetylcholine Containing an Acetylenic Bond
Equipotent Molar Ratios Relative to Acetylcholine*

	Dog blood pressure (fall)		Rabbit intestine
	Normal	After Neostigmine	
$CH_3COOCH_2C{\equiv}CCH_2\overset{+}{N}Me_3$	0 031–0 12	2 5	1 2
$CH_3COOCH_2CH_2C{\equiv}CCH_2\overset{+}{N}Me_3$	0 25–0 63	—	6 3
$CH_3COOCHMeC{\equiv}CCH_2\overset{+}{N}Me_3$	0 25–0 63	—	2 5
$(\pm)$-$CH_3COOCHMeCH_2\overset{+}{N}Me_3$ (*Mecholyl*)	0 2	4 0	—
$H_2NCOOCH_2CH_2\overset{+}{N}Me_3$ (*Carbachol*)	0 2	4 0–8 3	—
F 2268	0 031–0 12	5 0	—

Jacob, Marszak, Bardisa, Marszak Flairy, and Epsztein (1952)

Hexamethonium (Schoepke and Shideman, 1960) The most active appeared
to contain the unit, $-C{\equiv}C-CH_2\overset{+}{N}Me_3$ for the analogue (VII 8) of Hexa-
methonium the equipotent molar ratio relative to acetylcholine was about 2 5

$$Me_3\overset{+}{N}CH_2CH_2CH_2-C{\equiv}C-CH_2\overset{+}{N}Me_3$$

VII 8

Furmethide and 5-Methylfurmethide

Fellows and Livingston (1940) found muscarine like activity in 2 furfuryl-
trimethylammonium (*Furmethide*, VII 9) From a study of the compounds

Furmethide, VII 9

known to possess muscarine-like activity, Ing (1949) concluded that the length
of the chain attached to the onium group was of very great importance and
was optimal when it was comparable with the 'five atoms' (ignoring hydrogen)
in acetylcholine or peotyltrimethylammonium Accordingly, he thought it
likely that the activity of *Furmethide* could be increased by lengthening the
molecule and this expectation was fulfilled, *5-methylfurmethide* was consider-
ably more active (Table VII 6), being comparable with acetylcholine

F 2268 and F 2581

A similar variation of activity with chain length was found in a series of
highly active compounds, 1 3 dioxolanes, studied by Fourneau, Bovet,
Bovet, and Montezio (1944) Activity was maximal in the methyl member of

the series (F 2268; VII 10, Table VIII 8) The higher members of the series were antagonists (Van Rossum and Ariëns, 1959), and the effects of replacing methyl groups in the cationic head by ethyl groups were similar to those observed when similar changes were made to acetylcholine

F 2268, VII 10

Estimates of the activity of F 2268 vary considerably. On the cat blood-pressure Carr and Riggs (1951) found that acetylcholine, acetyl-β-methyl-choline, and F 2268 were all about equiactive, but on the dog blood-pressure and intestine and on bronchial muscle of the guinea-pig, Salle and Valade (1954) reported that comparable effects were produced by doses of F 2268 which were only 1 or 2 per cent of those of acetylcholine In the estimation of

TABLE VII 8

Activity of F 2268 and Analogues Equipotent Molar Ratios Relative to Acetylcholine

R R'		Dog blood-pressure	Rabbit intestine	Guinea pig ileum	Rat jejunum (VR and A)
H H		0·5–2·0	10–50	20	160
H Me	(*F 2268*)	0 1–0 02	0 1	0 1	0 91
H Et		10	10	10	130
H Ph		inactive			antagonist
Me Me		—	—	—	1,400
Et Et		—	—	—	790
CH₃CH⟨O–CH₃ / O–HC–CH₂ṄMe₂		—	—	—	0 91
—ṄMe₂Et		—	—	—	2 7
—ṄMeEt₂		—	—	—	500
—ṄEt₃		—	—	—	7,900
Carbachol		—	—	—	3 1
Mecholyl		—	—	—	3 8

Note — All compounds tested as racemates

VR and A = Van Rossum and Ariëns (1959) Other results from Fourneau, Bovet Bovet, and Montezin (1944)

the muscarine like activity of any compound, the results may be profoundly affected by differences in the cholinesterase content of the tissues and of the stability of the compounds to this enzyme, nevertheless, estimates of the activity of *F 2268* relative to acetylcholine, appear to vary more than might be expected The compound contains two asymmetric centres and it is possible that the composition of the mixture may vary from batch to batch, even though the material is optically inactive Triggle and Belleau (1962), for example, found that samples may contain more of the isomers in which the methyl and quaternary ammonium groups are on the same side of the ring than of the isomers in which they are on opposite side (60 per cent of the *cis* as opposed to 40 per cent of the *trans*)

$$R-CH \begin{array}{c} O-CH_2 \\ \diagdown \\ \diagup \\ O-CH_2 \end{array} CH-NMe_3^+$$

R = Me, *F 2581*, VII 11,
R = H, *F 2580*, VII 12

Even higher activity on the blood pressure of the dog was observed in the compound *F 2581* (VII 11, Tsatsas, 1950) The equipotent molar ratio relative to acetylcholine was about 0 007 and 0 7 for the *desmethyl* compound *F 2580* (VII 12) The ratio for both compounds was 0 015 when they were tested for their ability to stop the dog's heart, but on rabbit intestine they were less active than acetylcholine, the ratio for both compounds being 7

Muscarine and Related Compounds

Although the ability of muscarine to produce effects like those of parasympathetic stimulation has been known since the work of Schmiedeberg and Koppe (1869) the chemical structure of the compound has only recently been worked out The early work was complicated by the supposed synthesis of muscarine (Schmiedeberg and Harnack, 1877), the 'synthetic muscarine' was subsequently shown to be the nitrite ester of choline (Ewins, 1914) In his classification of the actions of acetylcholine into muscarine-like and nicotine-like, Dale (1914) was aware of the differences between the two substances Choline nitrite is much less active than acetylcholine, Dale (1914) found the equipotent molar ratio relative to acetylcholine on the cat's blood pressure was around 100 The compound has appreciable nicotine-like activity and is not purely muscarine like

Muscarine contains 3 asymmetric carbon atoms The relative positions of the groups were ultimately established by X-ray analysis (Kögl, Salemink, Schouten, and Jellinek, 1957), and the absolute configuration was determined by a synthesis of (+) muscarine from glucosamine acid of known configuration (Hardegger and Lohse, 1957, Fig VII 2) The chemistry has been reviewed by Eugster (1960) and the pharmacology by Waser (1962)

Natural (+)-muscarine is 2S-methyl-3R hydroxy tetrahydrofuryl 5S-methyltrimethylammonium (VII 13) The methyl group is on the same side of the ring as the onium group and *trans* to the hydroxyl group In *epi*muscarine (VII 14) all three groups are on the same side of the ring, in *allo*muscarine

FIG VII 2 *Synthesis of (+) muscarine from glucosaminic acid of known configuration (Hardegger and Lohse, 1957)*

(VII 15) the hydroxyl group and the onium group are *cis* and the methyl *trans*, and in *epiallo*muscarine (VII 16) the methyl and hydroxyl groups are *cis* and the onium group *trans* The activities of these compounds and of substances related to them are shown in Table VII 9

(+) 2S 3R 5S-Muscarine
VII 13

(+)-2S 3S 5S-*epi*muscarine
VII 14

(+)-2S 3R 5R-*allo*muscarine
VII 15

(+)-2S 3S 5R *epiallo*muscarine
VII 16

Values for the equipotent molar ratio of (+)-muscarine relative to acetyl choline on preparations containing postganglionic cholinergic receptors vary over about a 50-fold range, from 0 098 on the dog bladder to 5 4 in some experiments on the frog heart This variation could, of course, as with F 2268 (page 202), indicate changes in the sensitivity to acetylcholine well as changes in sensitivity to (+)-muscarine

Among the isomers and analogues of muscarine, marked activity is restricted to those compounds in which the arrangement of the methyl and hydroxyl groups and the onium side-chain is the same as in muscarine The most active of the other isomers is (±)-*epiallo*muscarine and in the (−)-form of this the hydroxyl group and the onium side-chain are in the same positions

TABLE VII.9

Activity of Compounds Related to Muscarine: Equipotent Molar Ratios Relative to Acetylcholine

HO,H
Me,H ⟨   ⟩ H,CH₂NMe₃

	Me	HO	CH₂NMe₃	Cat blood-pressure (W)	Frog heart (W)	(VR)	Rabbit ileum (G and U)	Dog bladder (G and U)
(+)-Muscarine	↑	↓		0 32, 0 30*	4 3, 5 4*		0 33	0 098
(±)-Muscarine	↑	↓	(r)	0 75	9 7	6 3	1 0	0 27
(−)-Muscarine	↑	↓		32				
(±)-Epimuscarine	↑	↑	(r)	230	63,000		230	170
(±)-Allomuscarine	↓	↓	(r)	130	4,500		350	49
(±)-Epiallomuscarine	↓	↑	(r)	75	3,600	500	220	36
(±)-normuscarine		↓	(r)	580	2,300			
(±)-2-desmethylmuscarine	—	↓	(r)	79	9,500			
(±)-2-desmethylepimuscarine	—	↑	(r)	550	130 000			
(±)-2-methylmuscarine	↓	↓	(r)	650	1,200			
(±)-3-phenylmuscarine	↑	↓		0 48	13			
(±)-Acetylmuscarine	↑	↓		0 54				
(±)-4:5-dehydromuscarine	↑	=	(r)	0 76	61			
(±)-4:5-dehydroepimuscarine	↑	=	(r)	1 5	120			

Me,H ⟨   ⟩ H,CH₂NMe₃

	Me	CH₂NMe₃						
(+)-Muscarone	↓			0 23			0 15	0 051
(±)-Muscarone	↓	(r)		0 11	3 0		0 13	0 034
(−)-Muscarone	↓			0 076	2 3		0 063	0 017
(±)-allomuscarone	↑	(r)		0 23	6 1		0 28	0 076
(±)-normuscarone		(r)		120				
(±)-2-desmethylmuscarone	—	(r)		4 8	2,500			
(±)-4:5-dehydromuscarone	↑	(r)		0 11	3 0		0 50	0 16
(±)-1-thiomuscarone	↑	(r)		2 9	290			

* The first figure was obtained with the chloride, the second with the iodide.

Figures for the rabbit ileum and dog bladder were calculated using the ratios 0 33 and 0 098 respectively for (+) muscarine relative to acetylcholine obtained by Fraser (1957) The symbol (r) indicates a racemic mixture of which only one isomer is indicated. — indicates the absence of a group and = that it is flat because it is attached to a double bond

W = Waser (1958, 1962); VR = Van Rossum (1960); G and U = Gyermek and Unna (1958)

as in (+)-muscarine, only the position of the methyl group is different. The relative position of all three groups is clearly important, but whereas the hydroxyl and the onium groups are polar, and could influence activity by becoming bound to the receptors by electrostatic forces or a hydrogen bond, the methyl group could only be bound by van der Waals' forces, though it may influence the activation of the ring oxygen atom. The dramatic effects on activity of altering the position of the methyl group can probably, therefore, best be explained by supposing that it (sterically) spoils the fit to the receptor when in the wrong position, although it does seem also to assist the fit when in the right position (i.e. as in muscarine). The equipotent molar ratio for (±)-2-methylmuscarine, for instance, is much greater than that for

(±)-2 *des*methylmuscarine (650 as compared with 79 on the cat) The activity of (±)-3 phenylmuscarine, however, is extremely interesting, because it indicates that in other parts of the molecule an appreciable increase in size does not necessarily reduce activity 1-Thiomuscarine, in which the oxygen atom of the tetrahydrofuran ring is replaced by a sulphur atom, is only feebly active This could be either because of the change in the size of the ring, which would alter the relative positions of the groups, or because of a change in the activation in the 1 position which might reduce attachment at that point (always supposing that this occurs at all)

With the muscarones activity is increased and there is a decline in stereospecificity The most disconcerting finding is that (+) muscarone, derived from (+)-muscarine (Eugster and Waser, 1957), and therefore having the same configuration, is less active than (−) muscarone, although the ratio of the doses producing similar effects is only about 3

The muscarones also possess some degree of nicotine-like activity This is consistent with what is known about compounds which stimulate the sites of the nicotine like actions of acetylcholine it could be ascribed, for example, to the presence of the partial positive charge on the carbon atom of the carbonyl group (or to the partial negative charge on the oxygen, if Sekul and Holland s view is accepted, see page 159) Consistent with this view is the finding that activity on the frog rectus is greatest in (±)-4 5 dehydromuscarine (equipotent molar ratio relative to acetylcholine, 0 11, for (−)-muscarone the ratio is 0 30 and for (+)-muscarone, 1 2) This is not a spectacular degree of activity, although it is enough to complicate tests for muscarine-like activity Herr and Gyermek (1960), for example, have found that (±)-muscarine is less active than acetylcholine in stimulating the mesenteric ganglion of the cat

Pilocarpine and Arecoline

Pilocarpine (VII 17) is a natural product which has long been known to have acetylcholine like activity It appears, however, to be only a partial agonist (Van Rossum, Cornelissen De Groot and Hurkmans, 1960) On rat intestine

Pilocarpine VII 17   Arecoline VII.18

it only gave 70 per cent of a maximal response, the equipotent molar ratio calculated from concentrations producing a 50 per cent response being 100 On the cat blood pressure the ratio was 300 Arecoline (VII 18), which occurs in the betel nut, is another natural product with similar feeble activity (Hunt and Renshaw, 1929)

Bis-onium Salts

In spite of the activity of polymethylene his trimethylammonium salts at the neuromuscular junction and at ganglia, these compounds have only slight

effects at postganglionic cholinergic receptors Paton and Zaimis (1949) recorded an equipotent molar ratio, relative to acetylcholine on guinea pig ileum of 430 for dodecamethylene bis trimethylammonium, the most active member of the series studied Polymethylene bis triethylammonium salts have some antagonist activity at postganglionic cholinergic receptors, but this is appreciable only in the members with very long chains, Warriner (unpublished) obtained an equipotent molar ratio relative to atropine on rat ileum of 11 for heptadecamethylene bis triethylammonium.

Barlow (1955) studied a series of polymethylene *bis* acetoxyethyldimethylammonium salts (VII 19) and observed a peak in activity at the decamethylene

$$CH_3\text{-}CO\text{-}O\text{-}CH_2CH_2\overset{Me}{\underset{Me}{\overset{+}{N}}}(CH_2)_n\overset{Me}{\underset{Me}{\overset{+}{N}}}CH_2CH_2\text{-}O\text{-}OC\text{-}CH_3$$

VII 19

compound, although this was not particularly active The equipotent molar ratios relative to acetylcholine on the frog heart were 1800, 26, 198, and 158 for the nona-, deca-, undeca , and dodeca-methylene compounds, respectively, and on the cat blood pressure the values were 700, 34, 470, and 460 respectively It was suggested that this indicated much greater affinity in the compound with the decamethylene chain, possibly because it became attached at both onium groups to two receptors at once Although it seems most unlikely that receptors are so close together that this is possible, it is quite conceivable that the second onium group becomes attached to an anchoring site, which is so placed relative to the receptor that the nonamethylene compound is too short to fit and the undecamethylene compound slightly too long If the chain in the undecamethylene buckles so that both onium groups are firmly held, there will be a decline in the contribution to adsorbability from Van der Waals' forces binding the chain to the surface lying between the receptor groups It may be questioned, however, whether the changes in activity really indicate changes in affinity and whether they may not be due at least in part, to changes in efficacy, for instance inability to cause depolarization.

Relationships Between Structure and Stimulant Activity

Estimates of the equipotent molar ratio relative to acetylcholine vary considerably from one preparation to another On the frog heart, for instance, there does not appear to be any compound which is more active than acetylcholine, whereas on the dog blood pressure many compounds, acetoxybut 2 ynyltrimethylammonium (VII 20, Table VII 7) F 2268 and F 2581, are

$$CH_3\text{-}C\overset{O}{\underset{O\text{-}CH_2\text{-}C\equiv C\text{-}CH_2\text{-}\overset{+}{N}Me_3}{\diagdown}}$$

VII 20

very much more active Activity on this preparation however is not always associated with comparable activity on the cat blood pressure or the intestine

and it is, accordingly, difficult to decide which compounds are the most active when they have not all been compared on the same preparations The results also emphasize that the structure of the receptors may not be exactly the same in all the preparations Nevertheless, the compounds most worthy of consideration appear to be acetoxybut-2-ynyltrimethylammonium, *F 2268*, *F 2581*, (+)-muscarine and (−)-muscarone

Alles (1939) and Ing (1949) have suggested that muscarine-like activity is primarily dependent upon the presence of the onium group and on the size of the molecule, there should not be more than five large atoms (carbon or oxygen) attached to the onium group, though in cyclic compounds allowance must be made for the effects of the ring on the distance between the onium group and the end of the chain Although this rule can be applied successfully to many compounds, including alkyltrimethylammonium salts, esters and ethers of choline, *F2268* and *F2581*, (+)-muscarine and (−) muscarone, it breaks down over 3-phenylmuscarine, (+) acetylmuscarine, and acetoxybut-2-ynyltrimethylammonium

A simple explanation for the exceptions might be that the size of the molecule need not be limited in all directions The 3-phenyl group in 3 phenyl-muscarone might be regarded as being inclined away from the surface to which the muscarine molecule is attached, and so not effectively increasing the length, but the acetyl group in (+)-acetylmuscarine could not be so regarded (only one isomer has been studied, it would be interesting to know if the (−)-isomer is also active) It would have to be supposed, therefore, that the receptors will tolerate an increase in length in this part of the molecule without loss of activity, whereas it will not tolerate an increase in chain length beyond the equivalent of five carbon atoms in the region corresponding to the 2-methyl group of muscarine It would further have to be supposed that most molecules become attached so that the chain is in a position corresponding with the 2-methyl group in muscarine This is fairly easy to imagine for ethers, esters, *Furmethide*, and *F 2268* Acetoxybut-2-ynyltri-methylammonium, and possibly the acetylene analogue of Hexamethonium (VII 8, page 201), might be regarded as exceptions, which, perhaps because of the linearity of the but-2-ynyl group, become attached so that the chain occupies a position equivalent to the substituents on the 3-position of muscarine

It seems unlikely, however, that activity depends only on the size of the molecule The electron distribution should surely be important if the observed variations of activity with structure are to be explained Stereospecificity, such as is observed with muscarine, implies attachment to the receptors at three points at least The moderate degree of stereospecificity observed in the muscarones, however, suggests that the attachment at one point contributes less to activity than attachment at the others, and possibly that the three points lie almost in line

The structure of postganglionic cholinergic receptors has been described by Hunt (1926) as a 'mosaic' and Lands (1951) has suggested that it is a 'trough'. As a consequence of the advances which have been made in the

stereochemistry and pharmacology of compounds related to muscarine, it is possible to obtain a more detailed picture (Becket, Harper, Clitherow, and Lesser, 1961; Waser, 1961, 1962) The points at which muscarine could become attached to the receptor are the cationic head, the ether oxygen atom, the 2-methyl group, and the 3-hydroxyl group Both the ether and hydroxyl oxygen atoms might be involved in hydrogen bond formation, but with the ether oxygen atom, as opposed to the hydroxyl oxygen atom, the receptor group must contain the hydrogen atom Binding by electrostatic forces might also occur to a lesser extent (as an alternative to hydrogen bonding) at the ether oxygen atom and the 3-hydroxyl group The 2-methyl group could be attached by Van der Waals' forces or could exert an inductive effect on the ether oxygen atom, but neither of these possibilities (especially the latter) seems to account entirely for the importance of the 2-methyl group (cf muscarine and *des*methylmuscarine) and for the need for it to be in the right position (cf (+)-muscarine and (−)-*epi*allomuscarine)

Waser (1961, 1962) has suggested that the binding of the ether oxygen atom is likely to involve hydrogen bonds rather than electrostatic attraction, and support for this is found in the relative inactivity of thiomuscarine derivatives (which would be less able to form hydrogen bonds) Beckett, Harper, Clitherow, and Lesser (1961) suggest that the receptor contains a partial positive charge at this point to 'accommodate the ether link of muscarine or the ester link of acetylcholine' Beckett, Clitherow, Harper, and Lesser also postulate another partial positive charge further away to 'accommodate the hydroxyl group of muscarine, the carbonyl group of acetylcholine, or double bond of the furan analogues of muscarine'. If the carbonyl group in acetylcholine and the hydroxyl group in muscarine are attached to the same receptor group, the attachment of one of these, if not both, must involve electrostatic forces rather than a hydrogen bond, because the hydroxyl group contains a hydrogen atom, whereas the carbonyl group does not, and could only form a hydrogen bond if the receptor group contains a hydrogen atom

Whatever may be true for acetylcholine, it seems that the attachment of the carbonyl group in muscarone is different Waser (1961, 1962) has pointed out that in (−)-muscarone the carbonyl group can be regarded as being placed relative to the cationic head in much the same way as the ether oxygen in muscarine (Fig VII 3) The attachment of muscarone might, then, be at the cationic head and by a hydrogen bond involving the keto group, which should be a stronger hydrogen bond than that binding the ether oxygen atom in muscarine itself The ether oxygen atom in (−) muscarone should only be important in so far as it may furnish some attachment, presumably electrostatic, at the receptor binding the hydroxyl group in muscarine Support for this idea comes from the observation that thiomuscarones (in contrast to thiomuscarines) are reasonably active It would be interesting to have the methylene analogue, 1 methyl 2 oxo-*cyclo*pentyl-4-methyltrimethylammonium (VII 21)

The attachment of the choline part of acetylcholine to the receptors seems to be clearly related to that of (+)-muscarine In muscarine there appears

to be considerable steric hindrance between the methyl groups of the cationic head and the hydrogen atom in the 5-position of the tetrahydrofuran ring (Barlow, 1960) A somewhat similar situation is observed in esters of β methyl-choline and, by analogy with (+) muscarine, workers in several laboratories thought it likely that the active (+)-isomer should have the S-configuration (Fig VII 4) some time before this was actually shown to be correct by Ellenbroek and Van Rossum (1961) The ether oxygen atom in the ester group must be analogous to the ether oxygen atom in (+)-muscarine, the β-methyl group contributes little to activity (cf acetyl-β methylcholine and acetylcholine) If the methyl part of the acetyl group is analogous to the 2 methyl group in muscarine, the carbonyl oxygen must be in a position close to that occupied by the 3-hydroxyl group in (+)-muscarine As acetyl-

FIG VII 3   *Hypothetical structure of acetylcholine receptor on smooth muscle*

choline is distinctly more active than the ethyl ether of choline, this carbonyl oxygen must contribute considerably to activity though whether it is hydrogen-bonded or merely held by electrostatic forces is not clear

The recent elucidation of the stereochemistry of F 2268 by Triggle and Belleau (1962) and Belleau and Puranen (1963) has shown that the stereo-specificity of this compound is strictly comparable with that of muscarine, rather than with that of muscarone The *cis* isomers, in which the methyl and quaternary ammonium groups are arranged as in (+) and (−)-muscarine, are more active than the *trans* and the (+)-form of the *cis* isomer, which has the 2S,4S configuration and is structurally comparable with (+)-muscarine, is about 100 times as active as the (−) 2R,4R- isomer

Among the most difficult results to fit into the picture is the feeble activity of the aliphatic ketones related to acetylcholine (Table VII 6) There appears

to be no reason why 4-oxo-*n* pentyltrimethylammonium should not fit the receptors in a manner analogous to (−)-muscarone, yet although this appears to be the most active of the ketonic analogues of acetylcholine, it is not a particularly potent compound The pictures that can be made of the receptor (e g Fig VII.3), however, whatever their obvious shortcomings, suggest further work which might be profitable and lead to further information about the structure of the receptors

FIG VII 4   (+)-*S Acetyl β methylcholine compared with* (+)-2*S* 3*R* 5*S muscarine*

It must be pointed out that the foregoing discussion takes no account of the different effects of structure on affinity and on efficacy As will be seen below, increases in affinity are often associated with decreases in activity, or of increases in antagonist activity It seems likely, therefore, that changes in structure which lead to decreased activity decrease efficacy, but it does not necessarily follow that changes which lead to increased activity do so only because they increase efficacy, some increase in affinity might be possible without loss of efficacy

ANTAGONISTS

Antagonists Developed from Partial Agonists

There is no need, with these compounds which stimulate postganglionic cholinergic receptors, to consider the blocking activity of agonists, as is necessary for compounds, such as nicotine, affecting the receptors at the neuromuscular junction or in ganglia There are, however, several groups of antagonists which have been derived from agonists, being members of a series in which, with increasing substitution, agonist activity is replaced by partial agonist activity and eventually antagonist activity

The simplest examples are the alkyltrimethylammonium salts already referred to (pages 194) Although agonist activity declines above *n* pentyltrimethylammonium, affinity increases in a fairly regular manner (Table VII 10) In the results of Stephenson, the value log K_{n+1}/K_n, for homologues with n and $n + 1$ carbon atoms, is around 0 2 If the relationship between the association constant K, and the free energy change on adsorption ΔG, is given by the theory of Arrhenius (as is usually assumed in work with enzymes, see, for instance, Dixon and Webb, 1958)

$$\Delta G = -RT \ln K$$

and hence

$$\log_{10} K_{n+1}/K_n = \frac{a}{2\ 3RT}$$

where a is the contribution to the free energy of adsorption ascribable to the extra methylene group in the longer homologue For the receptors on the guinea pig ileum the value $a = 2 \cdot 3 \times 1 \cdot 98 \times 310 \times 0 \cdot 2 = 280$ cals This would appear to be an answer reasonably consistent with Van der Waals' adsorption

TABLE VII 10

Antagonist Activity of Alkyltrimethylammonium Salts

$$R\overset{+}{N}Me_3$$

Log affinity constant (K_S)

	Guinea pig ileum (S)	Rat jejunum (VR and A)
R = n Heptyl	4 61	4 6
n Octyl	4 80	5 0
n-Nonyl	5 04	5 0
n-Decyl	5 28	5 9
n-Dodecyl	—	6 0

S = Stephenson (1956)   VR and A = Van Rossum and Ariens (1958)

Somewhat similar results have been observed in the series of aliphatic esters of choline studied by Schneider and Timms (1957, Table VII 11) Affinity increases in a fairly regular fashion up the series until a maximum is reached beyond which it declines relatively rapidly, probably because of the physical properties of the compounds, e g surface activity

TABLE VII 11

Activity of Esters of Choline as Antagonists of Acetylcholine on the Guinea pig Ileum

$$CH_3(CH_2)_n COO—CH_2CH_2\overset{+}{N}Me_3$$

	pA₂
n = 5 (Heptanoylcholine)	4 7
6	4 7
7	5 0
8	5 5
9	6 0
10	6 5
12	6 8
14 (palmitoylcholine)	6 4

Schneider and Timms (1957)

Quantitative results are also available for the rather more complicated compounds, analogues of F 2268, studied by Van Rossum and Ariens (1959) A decline in activity can be brought about either by substitution of large groups on the onium atom or by increasing the chain length of the acetal part of the molecule Van Rossum and Ariens have suggested that substitution at either point leads to increased affinity, but the results (Table VII 12)

TABLE VII 12

Antagonist Activity of Analogues of F 2268

$$R \diagdown \begin{array}{c} O - CH_3 \\ C \\ \end{array}$$

$$R \diagup \begin{array}{c} O - CH \cdot CH_2 \overset{+}{N}R_3 \end{array}$$

		pA₂ values on rat jejunum			
R =	R =	$R_3 = Me_2$	Me_2Et	$MeEt_2$	Et_3
H	Me	—	—	—	3 6
	Et	—	—	4 6	4 2
	n-Pr	—	4 9	—	4 8
	n Bu	4 9	—	—	—
	n-Hex	4 5	—	—	—
	Ph	4 1	—	—	—
R = R =					
	Me	—	—	—	—
	Et	—	4 9	—	—
	n Pr	6 2	6 8	6 9	6 6
	n Bu	6 9	7 2	—	—
	Ph	7 1	—	—	—

Note – The lower homologues are agonists or partial agonists

Van Rossum and Ariens (1959)

suggest that alterations to the acetal group have a bigger effect upon affinity than alterations to the onium group The atropine like properties of 2 2 diphenyl 1 3 dioxolane-4-methyltrimethylammonium (VII 22) were noted by

$$Ph \diagdown \begin{array}{c} O - CH_2 \\ C \\ \end{array}$$

$$Ph \diagup \begin{array}{c} O - CH - CH_2 NMe_3 \end{array}$$

VII.22

Brown and Werner (1949) who tested the compound because of its resemblance to antagonists of acetylcholine rather than because it resembled *F 2268* These results indicate an equipotent molar ratio relative to atropine on rat jejunum of about 10

The effects of substitution in the onium group of acetylcholine on affinity have been studied quantitatively by Scott (1962) By comparing the affinity constants of series of antagonists, $R\overset{+}{N}Me_3$ $R\overset{+}{N}Me_2Et$, $R\overset{+}{N}MeEt_2$, $R\overset{+}{N}Et_3$ he has shown that, when R is diphenylacetoxyethyl, benzilyloxyethyl, and 2 2 diphenyletboxyethyl, affinity increases with the substitution of one or two ethyl groups and then declines, although the triethyl compound still has a higher affinity than the trimethyl (Table VII 13) The results for the 5 5 diphenyl 2 oxo n pentyl series are slightly different, and the relatively lower affinity of the triethyl compound could be ascribed to interaction between the ethyl substituents on the onium atom and the nearby keto

pounds on the central nervous system nothing like the same degree of stereospecificity is observed, both the R- and the S-compounds being active

The activity of compounds closely related to atropine is shown in Table VII 15 In cats hyoscine is apparently more active than hyoscyamine in

TABLE VII 15
Blocking Activity of Compounds Related to Atropine

	Equipotent molar ratios relative to atropine					
	Cat saliva tion	Cat blood pressure	Cat eye	Mouse eye	Guinea-pig ileum	log K_B
(−)-Hyoscyamine	0.56	0.53	0.5	0.54	0.31	9.35
(+)-Hyoscyamine	11	12	—	10	10	7.85
Methylatropinium (*Eumydrine*)	0.48	0.54	1-2	0.44	0.59*, 0.47	
(−)-Metho-hyoscyaminium	0.25	0.25	1	0.20	—	
(−)-Hyoscine	0.73	0.83	0.07-0.1	0.20	1.3	
(−)-Methohyoscinium (*Pamine*)	0.29	0.29	0.3	0.21	0.17	

* Rabbit ileum.

K_B affinity constant derived from measurement of pA_x (page 43) in these experiments the pAs for atropine was 8.84 (cf pages 188, 194)

Nyman (1944) Bulbring and Dawes (1945) Ing Dawes, and Wajda (1945) Marshall (1945) Luduena and Lands (1954) Long Luduena Tullar and Lands (1956)

dilating the pupil of the eye, but slightly less active in stopping salivation and in antagonizing the effects of acetylcholine on the blood pressure Quaternization increases the activity of atropine and hyoscine, but the activity on the eye does not always run parallel with the activity in blocking salivation or the effects of acetylcholine on the blood pressure

At the postganglionic cholinergic receptors in the intestine hyoscine appears to be less active than atropine (particularly when it is remembered that hyoscine is tested as the naturally occurring (−) isomer whereas atropine is the racemate) Activity is very greatly enhanced by methylation, however, methylhyoscinium salts being very active indeed Quaternization with larger alkyl groups, such as n butyl, reduces ability to block postganglionic cholinergic receptors, but this particular compound, n butyl hyoscinium (*Buscopan*, Wick, 1951, VII 25) has considerable ganglion blocking activity Another compound, b biphenylylmethyl (+) tropyl x tropinium (*Gastropin*, VII 26, Gyermek and Nador, 1957) combines appreciable blocking activity at postganglionic cholinergic receptors (the equipotent molar ratio relative to atropine on rabbit intestine is 2.9) with appreciable ganglion blocking activity (page 162), much greater than that of *Buscopan* It does not have much effect on salivation, on the eye, or on the heart, but in vivo on intestine and

on the urinary bladder its effects are comparable in intensity with those of methylatropinium

Bis-atropinium derivatives, in which two molecules of atropine have been

Buscopan VII.25

joined together by penta-, hexa-, octa-, and deca-methylene chains (VII 27, Kimura, Unna, and Pfeiffer, 1949; Kimura and Unna, 1950, Eckfield, 1959), have been found to have appreciable activity in blocking postganglionic cholinergic receptors, but the results are not complete enough to indicate

Gastropin, VII.26

how activity may vary with the length of the polymethylene chain The decamethylene compound appeared to be about twice as active on the heart as methylatropinium on a weight basis; the molecule has a molecular weight about twice that of methylatropinium, but also contains two atropine units The neuromuscular blocking properties of these compounds have been discussed on page 128

n = 5, 6, 8 and 10, VII.27

Derivatives of Pseudotropine

The isomer of atropine, tropyl ψ tropine (VII 28), was isolated by Liebermann and Limpach (1892), who quote the otherwise unpublished report of Liebreich that the compound has no mydriatic properties, but blocks the effects of stimulating the vagus and is half as active as atropine on the blood-pressure This suggests that the arrangement at the 3-carbon atom of the

tropine portion is not critical for ability to antagonize the actions of acetyl choline, although it may alter ability to reach the receptors in the eye Alternatively, the postganglionic cholinergic receptors in the eye may be slightly different from those elsewhere, e g in the salivary gland and intestine

More information would be welcome and it is possible to obtain this, to some extent, indirectly by examining the properties of other derivatives of ψ tropine Benzilyl ψ tropine (VII 29) was studied by Kreitmair (1936) and

$$R = PhCH \cdot \ \ \text{tropyl-}\psi\text{-tropine, VII.28,}$$
$$\quad\quad\quad |$$
$$\quad\quad CH_2OH$$

$$R = Ph_2COH \cdot \ \ \text{benzilyl-}\psi\text{ tropine VII 29}$$

found to produce effects comparable with similar doses or concentrations of atropine on salivation to cats and on rabbit intestine To produce comparable effects on the cat blood pressure or in cats' or rabbits' eyes, however, the amount of benzilyl ψ tropine required was ten times that of atropine The compound has local anaesthetic properties which may modify its effects on the eye possibly suggesting that it is a more active mydriatic than its activity in antagonizing the actions of acetylcholine would lead one to suppose It is difficult to ascribe its low mydriatic activity to inability to penetrate through the mucous surface to the receptors in the eye, because it is able to anaesthetize the cornea It really appears, therefore, that the receptors in the eye may well be slightly different from those in the salivary gland or intestine Gyermek (1953) has compared the benzoyl esters of tropine and ψ tropine, and quaternary compounds derived from them, for their ability to act like atropine on rabbit and guinea pig intestine The derivatives of ψ tropine were consistently less active, the equipotent molar ratio for the ψ compound relative to the isomeric tropine varied from 2 to 10

It appears, then, that the configuration of the 3 hydroxyl group in the tropine part of the molecule does have some influence on activity at the postganglionic cholinergic receptors (Gyermek and Nador, 1957), but the effects are much more marked on activity at the sites in the eye than elsewhere

Esters of Tropine

Pyman (1917) reviewed the activity of a large number of derivatives of tropine as estimated by their ability to produce mydriasis when applied to cats' eyes in concentrations of 1 to 2 per cent Both tropine and tropic acid were in active, as were esters of tropine with aliphatic acids, such as acetic, glycollic, lactic, tartaric, succinic, and fumaric acids Feeble activity was found in some

aromatic esters of tropine, such as those of benzoic and o- and m-hydroxy-benzoic acids, but not those of p-hydroxybenzoic or phthalic acids The beneficial effect of the benzene ring on activity, however, does not appear to depend upon its influence on the electronic activation of the ester group, because activity is also found in phenylacetyltropine and in many of its derivatives, such as mandelyltropine (Homatropine, VII 30), o-, m-, and p-methyl mandelyl, and o-, m-, and p-hydroxymandelyltropines As is found with the hydroxybenzoyltropines, o-hydroxymandelyltropine is more active than the m isomer and the p-compound is least active

Homatropine is much weaker than atropine The equipotent molar ratios for the racemate relative to atropine are 7 7 on cats' eyes, 10 on the frog heart, and 6 1 on salivation in rabbits (Issekutz, 1917), 30 on salivation in dogs (Cushny, 1920), 7 9 on rabbit intestine, and 8 5 on guinea-pig intestine (Graham and Gunn, 1944) Brown and Quinton (1957) obtained a ratio of 100 for salivation in rabbits The effects on the eye are much less persistent than those of atropine so Homatropine has been used extensively for examining the eye in ophthalmology The eye usually returns to normal within 24 hours, whereas the local application of atropine may paralyse accommodation and dilate the pupil for several days

R = Ph—CHOH—CO , Homatropine , VII 30 ,

R = Ph—CH—CO , hydratropyltropine , VII 31 ,
      |
     Me

R = Ph—COH—CO , atrolactoyltropine , VII 32 ,
      |
     Me

R = Ph₂CH—CO , diphenylacetyltropine , VII 33 ,

R = Ph—CH—CO— , VII 34

Cushny (1920) observed that the activity of Homatropine was much less stereospecific than that of atropine or hyoscine, on salivation in dogs, it appeared that the equipotent molar ratio for the (+)-isomer relative to the more active (—) isomer was as low as 2 These results all emphasize the importance for activity of the hydroxyl group in the tropic acid part of the molecule This is emphasized by the observation of Cushny (1926) that hydratropyltropine (VII 31), in which the hydroxyl group in tropic acid is replaced by hydrogen, is only feebly active, the equipotent molar ratio for the racemate relative to atropine on salivation in the dog being of the order of 200 Some activity on the eye, however, is shown by compounds in which the

hydroxyl group is replaced by chlorine or bromine (Pyman, 1917), and atrolactoyltropine ('Pseudoatropine', VII 32), in which the hydroxyl group is transferred to the α-carbon atom, is comparable with Homatropine

As with atropine and hyoscine, quaternization of esters of tropine often increases activity Methylhomatropinium (*Novatropine*), in particular, has been studied in some detail The equipotent molar ratio relative to atropine calculated from the results of Issekutz (1917) is 2 2 on cats' eyes, 0 94 on the frog heart, and 0 85 on salivation in rabbits Cahen and Tvede (1952) obtained results which give ratios of 2 4 on the eyes of mice, 1 4 to 2 3 on salivation in rabbits, and 7 2 on isolated rabbit intestine

Diphenylacetyltropine (VII 33) and phenyl-α-thienylacetyltropine (VII 34) are not particularly active, but are of interest because of their relationship to other esters, such as diphenylacetylcholine (see page 213), and because of the high activity of quaternary salts derived from them Lands (1951) found the equipotent molar ratios relative to atropine on isolated rabbit intestine and intact dog intestine were 14 and 17 respectively for the diphenylacetyl ester, and 3 8 and 8 5 respectively for the phenyl-α-2-thienylacetyl ester For metho-salts of the latter, however, the ratios were 1 6 and 0 24 respectively, indicating considerable activity, particularly on the dog intestine

Esters of Other Amino-alcohols

The tropine ring structure is not essential for atropine like activity As long ago as 1897, Harnies observed that the mandelic ester of 1 2 2 6-tetramethylpiperidin-4-ol (*Eucatropine*, VII 35, cf β-*Eucaine*, page 58) was a

RN = MeN *Eucatropine*, VII 35,

RN = $Me_3\overset{+}{N}$ Methyleucatropinium VII 36

mydriatic The equipotent molar ratios relative to atropine for antagonizing parasympathetic effects on salivation and blood-pressure in cats are 29 and 42, and for mydriasis in mice the ratio is 250 (Bülbring and Dawes, 1945, Ing, Dawes, and Wajda, 1945) *Methyleucatropinium* (VII 36) is more active, the ratios being 1 6 on salivation, 14 on the blood pressure, and 36 on the eye *Eucatropine* contains two asymmetric centres and is a mixture of isomers, the configuration of these *is not known*, but in view of the low stereospecificity of Homatropine and of the small difference between the activities of the two forms of the methiodides of the benzilic esters of 1 2 2 6-tetramethylpiperidin-4-ol (Ing, Dawes and Wajda, 1945, see below), it seems unlikely that there are any great differences between the activities of the different isomers

Von Braun and Räth (1920) prepared some tropic esters of ω-hydroxy-

alkyl*nor*tropanes (VII 37), in which the ester group is attached at the end of the polymethylene chain introduced in place of the methyl group on the nitrogen atom, instead of as in the 3-position They record the otherwise unpublished report of Pohl, that the ester of 2-hydroxyethyl*nor*tropane (VII 38) is a mydriatic, but the compounds with a longer chain, esters of 3-hydroxypropyl- and 5 hydroxypentyl*nor*tropanes, are inactive

VII 37,
n = 2, VII 38

Von Braun, Braunsdorf, and Räth (1922) showed that even simpler structures, tropic esters of hydroxyalkyldialkylamines (VII 39) have mydriatic properties Esters of hydroxyethyldialkylamines were more active than those of hydroxypropyldialkylamines, and piperidino derivatives (VII 40) were

$$Ph-CH-CO-O-(CH_2)_n-NR_2$$
$$\overset{|}{CH_2OH}$$ VII 39,

  , VII 40

more active than derivatives of diethylamine or dimethylamine A similar compound, the tropic ester of 1-diethylaminoneopentan-3-ol (*Syntropan*, VII 41, Fromherz, 1933, 1936), is of interest because doses which affect

$$Ph-CH-CO-O-CH_2-\overset{Me}{\underset{Me}{\overset{|}{\underset{|}{C}}}}-CH_2NEt_2$$
$$\overset{|}{CH_2OH}$$

Syntropan, VII 41

the intestine have little action on the eye or the heart For this reason it was selected as being likely to be a good spasmolytic, reducing the painful contractions of the gut in colic, for instance, without disturbing the rest of the body *In spite of its use as a spasmolytic, however, it is relatively feeble* compared with atropine, the equipotent molar ratio on rabbit intestine being of the order of 20

Developments Based on Acetylcholine

Further developments in the preparation of substances which antagonize the actions of acetylcholine at postganglionic cholinergic synapses have come from the study of compounds related to acetylcholine itself, rather than by modification of known antagonists, such as atropine A number of substances related to acetylcholine, which are only partial agonists, have already been mentioned (page 211) Generally, efficacy is lost, but affinity retained (or even enhanced) by increasing the size of substituents in parts of the molecule such as the onium group or the acyl group

TABLE VII 17

Activity of Analogues of Benzylylcholine Equipotent Molar Ratios Relative to Atropine

Ph₂C(OH)(COOCH₂CH₂-)	Mouse pupil	Cat salivation	Cat blood pressure	Rabbit intestine	Guinea pig ileum affinity constant*
NMe₂	77	91	10	—	—
NEt₂	17	56	48	—	—
+NMe₃	32	0.51	0.97	—	3.44×10^8
+NMe₂Et (*Lachesine*)	0.96	0.39	0.55	0.96	8.66
+NMeEt₂	16	0.37	0.42	—	8.98
+NEt₃	12	0.37	0.53	11	4.74
+NMe₂n-Pr	45	0.68	10	—	—
+NMe₂iso-Pr	11	0.34	0.42	—	—
+NMe₂CH₂CH=CH₂	36	—	—	—	—
+NMe₂n-Bu	91	13	16	—	—
Ph C(OH)(COO-)					
(CH₂)₂NMe Et	16	—	—	18	—
(CH₃)₂NMeEt₂	17	—	—	—	—
CH₂CMe₂CH₂NMe·Et	0.70	—	—	—	—
CH₂CH₂-N◯	200	—	—	—	—
CH₂CH₂-N◯Me	63	—	—	—	—
β-CH(CH₂-CHMe / NMe / CH₂-CMe₂)	63	—	—	—	—
β-CH(CH₂-CHMe / +NMe₂ / CH₂-CMe₂)	0.59	—	—	—	—
α-CH(CH₂-CHMe / +NMe₂ / CH₂-CMe₂)	0.71	—	—	—	—

Note – In the tests for mydriatic activity in mice the drugs were injected intraperitoneally and not applied direct to the eye. The letters α and β indicate differences in the configuration at the 4-position of the piperidine ring. *Eucatropine* (page 220) was in this work described as belonging to the β series

* *Scott* (1962)

Ing Dawes and Wajda (1945) *Ford Moore and Ing* (1947)

Further Esters of Amino-alcohols

It has already been pointed out that although *Syntropan* (page 221) is a reasonably specific spasmolytic, it is not particularly potent Meier (1936) observed very similar activity in the diphenylacetic ester of 2 diethylaminoethanol (*Trasentin, Adiphenine*, VII 44) Halpern (1938), who studied a large number of alkamine esters of disubstituted acetic acids, was particularly impressed with the properties of the α phenylvalerie ester of 2 diethylaminoethanol (*Propivane*, VII 45) The equipotent molar ratios relative to atropine are approximately 2 for antagonising the effects of acetylcholine on rabbit intestine, 40 on salivation in the rabbit, 200 on the frog heart, and 5,000 for mydriasis in cats produced by local application to the eye Like *Syntropan*, this compound appears to be more active at the postganglionic cholinergic receptors in the intestine than at those elsewhere, but it is, nevertheless, not particularly active

$$R\!-\!\!-\!\!-O\!-\!CH_2CH_2NEt_2$$

R= Ph$_2$CH—CO—, *Trasentin*, VII 44

R= CH$_3$CH$_2$CHPh—CO—, *Propivane*, VII 45,

R= PhCH—CO—, *Trasentin-H*, VII 46,

R= ⟨⟩ CO—, *Dicyclomine*, VII 47

The α phenyl α cyclohexylacetic ester of 2 diethylaminoethanol (*Trasentin H*, VII 46, Miescher and Hoffmann, 1941) appears to be more active Graham and Lazarus (1940) obtained results which give values of 1 2 for the equipotent molar ratio relative to atropine on isolated rabbit intestine and 4 0 for *Trasentin* itself In intact rabbits Trasentin H appeared to be more active than atropine, the ratio being about 0 5 These figures for *Trasentin* indicate greater activity than has been found by other workers Brown, Thompson, Klahm, and Werner (1950) obtained results on rabbit intestine which indicate an equipotent molar ratio for *Trasentin* relative to atropine of 80 For the 2 diethylaminoethyl ester of 1-cyclohexylcyclohexane-1 carb oxylic acid (*Dicyclomine*, VII 47) the ratio was 8 0, indicating that the compound is more active

The fluorene 9-carboxylic ester of 2 diethylaminoethanol (*Pavatrine*, VII 48) appears to have rather similar activity Lehmann and Knoefel (1942) obtained a value of 7 1 for the equipotent molar ratio relative to atropine on rabbit intestine and in the same experiments obtained values of 43 for *Trasentin*, 94 for *Syntropan*, and 55 for *Propivane* Brown, Thompson, Klahm, and Werner (1950) obtained the value of 8 0 for *Pavatrine* Pavatrine had only feeble activity on the eye, on salivation and on blood vessels and unfortunately was not as active *in vivo* on the gastrointestinal tract as might have been expected from the experiments on isolated tissues The equipotent molar ratio relative to atropine was about 20 for ability to reduce gastric

motility in dogs and greater than 100 for delaying gastric emptying and reducing gastric secretion The activity of *Pavatrine* relative to *Trasentin* in these results agrees well with the values obtained by Lands, Hoppe, Lewis, and Ananenko (1950) in which it was observed that the equipotent molar ratio for *Trasentin* relative to *Pavatrine* was 6 0 (cf 43/7 1 = 6 1)

R = H *Pavatrine*, VII 48,
R = OH, VII 49

The hydroxy derivative of *Pavatrine*, the ester of 9 hydroxy fluorene-9-carboxylic acid (VII 49), however, was slightly less active, whereas the hydroxy derivative of *Trasentin*, 2-diethylaminoethylbenzilate (*Benactyzine*, VII 50), was extremely active, about forty times as active as *Trasentin*, which would be equivalent to an equipotent molar ratio relative to atropine of 1 0 This may be an overestimate, because the compound was included in the work of Ing, Dawes, and Wajda (1945), and the results (Table VII 17) on the salivation and blood pressure do not suggest that it is particularly active

R' = Ph   NR₂ = NEt₂ , *Benactyzine* , VII 50 ,

R = ⬡ , NR₂ = ⁺NEt₂Me , *Oxyphenonium* , VII 51

The hydroxy analogue of *Trasentin-H* was found by Lehmann and Knoefel (1944) to have an equipotent molar ratio relative to atropine on rabbit intestine of 6 1 (unfortunately, *Trasentin-H* itself was not included in the same set of experiments), but activity is greatly increased by methylation The metho-salt, *Oxyphenonium* (*Antrenyl*, VII 51, Plummer, Barrett, Rutledge, and Yonkman, 1953), which is closely related to *Lachesine*, was as active as atropine on both isolated and intact preparations of intestine from guinea-pigs and dogs It was also effective to reducing gastric secretion in dogs and in reducing the formation of experimentally-induced ulcers in rats, it was, however, less active than atropine in producing mydriasis in rabbits and in preventing salivation in dogs Brown and Quinton (1957) found it to be slightly more active than atropine on salivation in rabbits

Lehmann and Knoefel (1944) also observed that the 2 diethylaminoethyl ester of xanthen 9-carboxylic acid (VII 52) was a potent antagonist of the actions of acetylcholine, on isolated rabbit intestine the equipotent molar

ratio relative to atropine was 3 4 (cf 7 1 for *Panatrine*) The xanthen portion can be replaced by phenothiazine as in the compound *Transergan* (VII 53), Wieldling (1957) obtained an equipotent molar ratio for this compound relative to atropine on the guinea-pig ileum of 2 1.

The quaternary metho-compound *Methanthelinium* (Banthine, VII 54, Hambourger, Cook, Winbury, and Freese, 1950) is more active, the equipotent molar ratio relative to atropine on isolated rabbit intestine was 1 2. It reduced salivary secretion, and caused mydriasis, and also had some

NR₂ = NEt₂, VII,52,

NR₂ = ṄEt₂Me, *Methanthelinium*, VII 54,

NR₂ = ṄIsoPr₂Me, *Propanthelinium*, VII 55

Transergan, VII 53

ganglion-blocking properties According to Barrett, Rutledge, Plummer, and Yonkman (1953) it was less effective than *Oxyphenonium* in reducing gastric secretion in dogs and experimentally induced ulcer formation in rats The di isopropyl analogue, *Propanthelinium* (Pro-Banthine, VII 55) appears to be slightly more active Johnson and Wood (1954) obtained equipotent molar ratios relative to atropine on the guinea-pig ileum of 0 40 for *Propanthelinium* and 0 48 for *Methanthelinium* On salivation in cats the values were 0 26 and 0 37, but for mydriatic activity in mice they were approximately 1 6 and 3 0, respectively

The 1-phenyl *cyclopentane*-1-carboxylic acid ester of 2-diethylaminoethanol (*Caramiphen, Parpanit*, VII 56, Domenjoz, 1946) also has appreciable

Caramiphen, VII 56

atropine like activity Tripod (1949) recorded equipotent molar ratios relative to atropine on rabbit ileum of 8 2 for *Caramiphen*, 19 for *Trasentin*, and 7 1 for *Trasentin-H*, on guinea pig ileum the values were 11, 57, and 2 9, respectively Domenjoz (1946) obtained a value of 12, but results with *Trasentin* in his experiments were different from those of other workers The action of *Caramiphen* at postganglionic cholinergic receptors is less important than its actions on the central nervous system, particularly in the suppression of the tremors and rigidity associated with Parkinson's disease

Another hydroxy derivative of *Trasentin-H*, the α-phenyl-α-(1-hydroxy)-*cyclo*hexylacetic acid ester of 2-diethylaminoethanol (VIII 57, Ehrenberg, Ramp, Blanchard, and Treves, 1952), appears to have about the same

activity as *Trasentin-H* itself and to be slightly more active than the 1-hydroxy-*cyclo*pentyl analogue (VII 58) The equipotent molar ratios relative to atropine on rabbit ileum were 3 3 and 3 9 respectively The metho-salts were much more active, the value for the compound (VII 59) analogous to *Oxyphenonium* was 0 39 and for the *cyclo*pentyl compound (VII 60) it was 0 75

$$R' = \text{(cyclohexyl-OH)}, \quad NR_2 = NEt_2, \quad VII.57,$$
$$, \quad NR_2 = \overset{+}{N}Et_2Me, \quad VII 59,$$
$$R' = \text{(cyclopentyl-OH)}, \quad NR_2 = NEt_2, \quad VII 58,$$
$$, \quad NR_2 = \overset{+}{N}Et_2Me, \quad VII 60$$

Extremely high activity has also been found in the 2-diethylaminoethyl ester of α-*cyclo*pentyl-α (2-thienyl)glycollic acid (VII 61) and its metho-bromide (*Penthienate*, *Monodral*, VII 62, Luduena and Lands, 1954) The equipotent molar ratios relative to atropine on rabbit ileum were 0 48 and 0 39 respectively, and 8 0 and 0 26 on salivation in rabbits The quaternary etho compound is also highly active (Lands and Luduena, 1956)

$$NR_3 = NEt_3, \quad VII 61$$
$$NR_3 = \overset{+}{N}Et_2Me, \quad \textit{Penthienate}, \quad VII 62$$

Esters of N-methylpiperidine-3-carbinol have been studied by Lands (1951), and the most active appears to be the metho-compound derived from the benzilic ester (VII 63) for which the equipotent molar ratio relative to atropine on rabbit intestine is 2 3 The ratio on dog intestine *in vivo* is 0 82 The N-ethyl analogue (*Piperzolate methobromide*, *Piptol*, VII 64, Chen and

$$\overset{+}{N}RR = \overset{+}{N}Me, \quad VII 63,$$
$$\overset{+}{N}RR' = \overset{+}{N}EtMe \quad \textit{Pipenzolate methobromide}, \quad VII 64$$

Beckman, 1952, Chen, 1954, 1959) is reported to be as active as atropine in relieving spasm of the ileum and colon of dogs It appears to be particularly effective on sphincters and on the colon The metho salt of the methyl ester of piperidine-3-carboxylic acid (VII 65) is less active (Lands, 1951) Acred, Atkins, Bainbridge, Brown, Quinton, and Turner (1957) have examined

similar 2-substituted pyrrolidine derivatives, and again the most active was a benzilic ester, that of 2-hydroxymethyl-NN-dimethylpyrrolidinium (VII 66, *Poldine, Nacton*) On the cat blood-pressure and salivary flow, and as a mydriatic in mice, this appeared to be about as active as atropine On the isolated guinea-pig ileum it was also as active as atropine, but was less active than some of the other compounds studied, particularly the ethiodide of the benzilic ester of 2-(2-hydroxy-ethyl)-N-methylpyrrolidine (VII 67)

R = Me n = 1, *Poldine*, VII 66
R = Et n = 2, VII 67

Considerable activity was also observed in esters of quinuclidin-3-ol (Randall, Benson, and Stefko, 1952) The equipotent molar ratios relative to atropine on rabbit intestine were 0 90 for the racemic form of the diphenyl-acetic ester (VII 68), 0 46 for the racemic form of the benzilic ester (VII 69), and 0 49 for the racemic form of the fluorene-9-carboxylic ester (VII 70)

R = Ph₂CH—CO—, VII 68,
R = Ph₂COH—CO— VII 69,
R =

VII 70

Activity was not increased by methylation, however, for the metho derivative of the diphenylacetic ester the ratio was 4 2 and for the metho derivative of the benzilic ester it was 0 80 The diphenylacetic ester of quinuclidin 3 ol was resolved, and it was found that the (—) isomer (ratio, 0 49) was much more active than the (+) isomer (ratio, 12)

Effects of Altering the Ester Link

The replacement of the ether oxygen atom in the ester link by a sulphur atom may increase atropine-like activity The thio analogue of *Trasentin*, the diphenylacetic ester of 2 diethylaminoethanthiol (VII 71) is appreciably more

$$R-S-CH_2CH_2NR_2'$$

R = Ph₂CH-CO-, R' = Et, VII 71,
R = Ph₂COH CO-, R' = Et, VII 72,
R = Ph₂COH CO-, R = Me, VII 73

active than Trasentin itself (Dupre, Levy, and Tchoubar, 1946, Ramsey and Richardson, 1947), and a number of analogous benzilic esters has been studied by Parkes (1955) The benzilic ester of 2 diethylaminoethanthiol

(VII 72) was more active than atropine on the guinea pig ileum (equipotent molar ratio, 0 82) and the ester of 2-dimethylaminoethanthiol (VII 73) was more active still (equipotent molar ratio 0 61) The activities of the oxygen analogues of these compounds (not, unfortunately, on guinea pig ileum) are included in Table VII 17 These thio-compounds are extremely active in antagonizing spasms produced by barium ions, which do not act on the acetylcholine receptors but possibly directly on the muscle-cells

Compounds in which the ester link is replaced by amide were studied by Meier and Hoffmann (1941) and appear qualitatively to resemble the esters, although they are not always as active Cheney and Bywater (1942), for example, found the 2 morpholinoethylamide of diphenylacetic acid (VII 74)

Ph
Ph—C—CO—NH—CH$_2$CH$_2$N $\bigcirc$ O

VII 74

Ph   C≡N
Ph—C—CH$_2$CH$_2$NR$_2$

VII 75

was less active than the ester The carbonyl group, in fact, does not appear to be critical and considerable activity has been found among substituted propylamines

Lands, Ananenko, Jones, Hoppe, and Becker (1949) observed that some dialkylaminoalkyl diphenylacetonitriles (VII 75) appeared to be as active as *Trasentin* and that some of the quaternary metho derivatives were almost as active as atropine (Table VII 18) Cunningham *et al* (1949) observed con-

TABLE VII 18
Activity of Diphenylacetonitriles

Ph   CN
Ph—C—R

	Equipotent molar ratio relative to atropine on rabbit intestine	
	Base	Metho-salt
R =		
CH$_2$CH$_2$NMe$_2$	125	40
CH$_2$CH$_2$NEt$_2$	62	4 8
CH CH$_2$N $\bigcirc$	125	3 5
CH CHMeNMe$_2$	20	4 1
CH$_2$CHMeN $\bigcirc$	77	1 9
CHMeCH$_2$NMe$_2$	53	67
CHMeCH$_2$NEt$_2$	140	50
Trasentin	42	—

Lands, Ananenko, Jones, Hoppe and Becker (1949)

siderable activity in an analogous propanol derivative, *Benzhexol (Artane,
VII 76)* The equipotent molar ratio relative to atropine on rabbit intestine
was 2 2, and for the diphenyl derivative, 1-piperidino-3 3-diphenylpropan-1-ol (VII 77), it was 2 8 This latter compound was also included in a
series of 3 3 diphenyl propanolamines, nllylamines, and propylamines and
their quaternary salts, studied by White, Green, and Hudson (1951) *The
allylamines and propylamines had little atropine like activity, but some of the
substituted propanols, particularly the quaternary derivatives, were quite
active* The equipotent molar ratio relative to atropine on rabbit intestine was
between 2 1 and 4 2 for 1-piperidino 3 3 diphenylpropan-1 ol (VII 77, cf

Ph OH

$$R-C-CH_2CH_2NR_2'$$

$R - \bigcirc$, $NR_2' - N\bigcirc$, *Benzhexol*, VII 76,

$R - \bigcirc$, $NR_2' - N\bigcirc$, VII 77,

$R - \bigcirc$, $NR_2' - \overset{+}{N}Et_2Me$, VII 78,

$R - \bigcirc$, $NR_2' - N\bigcirc$, *Procyclidine*, VII 79,

$R - \bigcirc$, $NR_2' - N\overset{+}{\underset{Me}{\bigcirc}}$, *Tricyclamol*, VII 80,

2 8 above) Similar series of compounds have also been studied by Lands
(1951) and Lands and Luduena (1956) who obtained equipotent molar
ratios relative to atropine on rabbit intestine of 3 4 for the diphenyl compound (VII 77), 5 1 for *Benzhexol* (cf 2 2 above) and 0 97 and 3 7 for its
metho- and etho-bromides, respectively for the *cyclopentyl* analogue of
Benzhexol the ratio was 3 3 The high activity of some of the quaternary
compounds was also noted by White, Green, and Hudson (1951), who obtained an equipotent molar ratio relative to atropine on rabbit intestine of
0 82 to 1 2 for the methyldiethylammonium compound (VII 78) This latter,
unlike the piperidino compounds, also had appreciable mydriatic activity,
the equipotent molar ratio relative to atropine in mice was 1 1 Structurally
this compound is distinctly reminiscent of *Lachesine*.

The pyrrolidine analogue of *Benzhexol, Procyclidine* (VII 79, Montuschi,
Phillips, Prescott, and Green, 1952) is less active, but the quaternary metho
derivative, *Tricyclamol* (VII 80, Lee, Gibson, Dinwiddie, and Mills, 1954)
is very active These 3 phenyl 3 *cyclohexyl* compounds all contain an asymmetric carbon atom and Duffin and Green (1955) have compared the activity
of the optical isomers The results (Table VII 19) show that there is a high
degree of stereospecificity, the (−) isomer being considerably more active
than the (+) The results also indicate the high activity of *Tricyclamol*, the
equipotent molar ratio relative to atropine on guinea pig ileum being 1 2 for
the racemate This compound, like some of the other quaternary salts, has
appreciable activity on the eye and has some ganglion blocking activity

TABLE VII 19
Activity of Optical Isomers

			Equipotent molar ratio relative to atropine			
			Guinea-pig ileum	Stereospecific index	Mydriasis in mice	Stereospecific index
N⌷	*Procyclidine*	(±)	2 2		3 1	
		(+)	54 0	49	>32 0	>1 8
		(−)	1 1		1 8	
Me⁺N⌷	*Tricyclamol*	(±)	1 2		2 3	
		(+)	8 1	160	8 1	62
		(−)	0 51		1 3	
Et⁺N⌷		(±)	2 0		2 0	
		(+)	23 0	290	20 0	200
		(−)	0 79		1 0	
N⬡	*Benzhexol*	(±)	3 7		1 6	
		(+)	13 7	9 8	4 1	4 8
		(−)	1 4		8 6	
Me⁺N⬡		(±)	1 4		1 8	
		(+)	4 5	48	2 4	33
		(−)	0 94		0 73	

The stereospecific index is the number of molecules of the (+) isomer producing the same effect as one of the (−) isomers Long Luduena, Tullar, and Lands (1956) recorded a stereo-specific index of 160 for the isomers of *Benzhexol* on rabbit intestine and of 30 for the slightly less active 1 *cyclohexyl* 1-(2 thienyl)-3 piperidino-propan 1 ol

Duffin and Green (1955)

Another closely related compound, *Mepiperphenidol* (*Darstine*, VII 81), has been reported to have atropine-like activity on rabbit intestine comparable with that of *Methanthelne* (McManus, Bochey, and Beyer, 1953), but less than that of atropine itself It is extremely effective in reducing gastric secretion (McCarthy, Evans, Ragins, and Dragstedt, 1953), but this property may not be entirely ascribed to its ability to antagonize the actions of acetylcholine at the postganglionic cholinergic receptors, because the compound reduces gastric secretion in preparations in which the parasympathetic nerve supply to the stomach has been cut

Marked ability to reduce gastric secretion is also found in 4-diphenyl-

methylidene-NN-dimethylpiperidinium (*Diphemanil*, VII 82, Margolin et al, 1951; this compound is also called *Prantal*, but should not be confused with NN-dimethyl-N'-phenylpiperazinium, VI 21, a ganglion stimulant with the same name) The effects appeared to be comparable with those of *Methantheline*, but the compound was only about half as active It was not particularly active on isolated guinea pig intestine, the equipotent molar ratio relative to atropine being 8 0, and had virtually no mydriatic activity It had some ability to block transmission in ganglia

The compounds so far discussed can be divided into substituted propylamines, substituted 3-amino-propan-1-ols, and a few substituted 3-amino-*n*-butyronitriles

Mepiperphenidol VII 81

Activity has also been observed in molecules containing an amide link attached to the propyl chain, such as 4-dimethylamino-2 2 di phenyl *n*-valeramide (*Aminopentamide*, VII 83, Hoekstra and Dickison, 1950, Cazort, 1950, Hoekstra, Tisch, Rakieten, and Dickison, 1954) The equipotent molar

Diphemanil, VII 82

ratio relative to atropine on rabbit intestine is 2 1 Activity decreases upon methylation, for the metho salt the ratio is 20

De Jongh, Van Proosdij Hartzema, and Janssen (1955, Jageneau and Janssen, 1956) have examined many compounds of this type and observed considerable atropine-like activity, both on the intestine and on the eye, in the quaternary metho derivative of 4 di *iso*propylamino-2 2-diphenylbutyramide (*Priamide*, VII 84), the equipotent molar ratio relative to atropine on rabbit intestine was 0 85 and the base itself was much less active, the ratio

R = Me , NR′₂ = NMe₂ , *Aminopentamide*, VII 83,

R = H , NR′₂ = ṄisoPr₂Me , *Priamide*, VII 84,

R = H , NR′₂ = N⟨ ⟩ , VII 85,

R = H , NR′₂ = N⟨ ⟩Me , VII 86

being 12 Even higher activity was found in the methylpiperidinium analogue (VII 85, ratio, 0 41) and in the methylpyrrolidinium analogue (VII 86, ratio, 0 71), both these quaternary compounds being more active than the parent tertiary bases

A number of phenothiazine derivatives have been found to have some

atropine like activity The ester *Transergan* has already been mentioned (page 227) and the carbonyl linked compound *Secergan* (VII 87) appears to have similar activity, the equipotent molar ratio relative to atropine on guinea pig intestine being 1 9 (Wiedling, 1957) Other phenothiazines are less active For Promethazine (VII 88) and *Diethazine,* (VII 89) for instance, the ratios

R = CO—CHMeNMe₂ *Secergan* VII 87,
R = CH₂CHMeNMe₂ *Promethazine,* VII 88,
R = CH₂CH₂NEt₂ *Diethazine,* VII 89

were 4 1 and 22 respectively Similar results have been obtained by Domenjoz and Theobald (1959) with series of phenothiazines 1-alkyl-4 5 dihydro 2 3-6 7 dibenzazepines (such as *Imipramine,* VII 90) and 1 alkyl 2 3 6 7 di benzazepines The equipotent molar ratios relative to atropine on rabbit intestine were 94 for *Diethazine* 30 for Promethazine and 11 for *Ethopropazine,* which was the most active on the compounds studied on this pre-

Imipramine VII 90

paration The value for *Imipramine* was greater than 150 Many of these substances have ganglion blocking properties as well as depressant effects on the central nervous system which might make them of value in depressing *gastric secretion and motility in intact animals Many of them also antagonize* the effects of histamine, but this is unlikely to account for any effects on gastric secretion, because the action of histamine in causing secretion of gastric juice is unaffected by any of the antihistamine drugs so far known

Discussion

The most active compounds those with an equipotent molar ratio relative to atropine of less than 1, are listed in Table VII 20 Although it is very difficult to justify collecting together the results from such a variety of experiments in this way, it seems likely that the Table gives some idea of the relative activity of the compounds Figures obtained for some of the compounds by direct comparison in a fairly comprehensive test of atropine-like spasmolytics by Wiedling (1957) are shown in Table VII.21 These agree reasonably

TABLE VII 20

Summary

Activity of Antagonists at Postganglionic Cholinergic Receptors in Guinea pig Intestine

	Equipotent molar ratios relative to atropine
(−)-Methohyoscyaminium	0 47
(−)-Methohyoscinium	0 17
(±)-α Phenyl-α thenylacetyltropine (VII 34)	0 24 (r)
(Benzyloyloxyethyl)ethyldimethylammonium (VII 43)	0 96 (r)
(Benzyloyloxyethyl)methyldiethylammonium	0 8–1 6*
(±)-(α Phenyl-α *cyclohexyl*glycoloyloxyethyl)methyldiethyl ammonium (VII.51)	1 0
(Xanthenyl 9-carboxyethyl)methyldiethylammonium (VII 54)	0 48
(Xanthenyl 9-carboxyethyl)methyldi *isopropyl*ammonium (VII 55)	0 40
(±)-(α Phenyl-α 1 hydroxy*cyclohetyl*carboxyethyl)methyl diethylammonium (VII 59)	0 39 (r)
(±)-(α-*cyclopentyl*-α 2 thenylcolloyloxyethyl)methyl diethylammonium (VII 62)	0 39 (r)
(±)-3 Benzoyloxyquinuclidine (VII 69)	0 46 (r)
Benzyloylthioethyldiethylamine (VII 72)	0 61
(±)-(1 Phenyl 1-*c*yclohexyl 1 hydroxy-*n* propyl)methyldiethyl ammonium (VII 78)	0 82–1 2 (r)
(−)-(1 Phenyl 1 *cyclohexyl* 1 hydroxy *n* propyl)methylpyrrolidinium (VII 80)	0 51
(−)-(1 Phenyl 1-*cyclohexyl* 1 hydroxy *n* propyl)ethylpyrrolidinium (VII 84)	0 79
(1 1 Diphenyl 1-carbamoyl *n* propyl)methyl-di *isopropyl* ammonium (VII 84)	0 85 (r)
(1 1 Diphenyl 1-carbamoyl *n* propyl)methylpiperidinium (VII 85)	0 4 (r)
(1 1 Diphenyl 1-carbamoyl *n* propyl)methylpyrrolidinium (VII 86)	0 71 (r)

* Calculated from estimates of K_B

(r) indicates experiments on rabbit intestine

TABLE VII 21

Activity of Antagonists at Postganglionic Cholinergic Receptors in Guinea pig Intestine

	Equipotent molar ratios relative to atropine
Syntropan (VII 41)	18
Pavatine (VII 48)	5 0
Trasentin H (VII 46)	2 0
Caramiphen (VII 56)	4 6
Methanthelinium (VII 54)	0 83
(−)-Hyoscine	0 40
(−)-Methohyoscinium	0 30

Wiedling (1957)

satisfactorily with estimates for the same compounds shown in Table VII 20 (Wiedling's results were not included in this Table)

Atropine itself appears to be a highly active substance Although several compounds are more active than it, even the strongest of them, N-methylhyoscinium, is not more than ten times as powerful Few of the compounds, in fact, are more potent than (−)-hyoscyamine

Most of the compounds in Table VII.20 are quaternary salts, and as a general rule it appears that quaternization with a small alkyl group increases activity The chief exception to this generalization seems to be the metho derivative of (±)-3-benziloyloxyquinuclidine

Examples of the relative activity of stereoisomers are given in Tables VII 14 and VII 19 It is remarkable that in all the instances where resolution has been achieved and the individual isomers tested, the (−) isomer is invariably more active While it is reasonable that the active isomers should all be related to (−)-S-hyoscyamine, it is surprising that in such a variety of compounds these should all have the same sign of rotation

The extreme stereospecificity exhibited by atropine-like antagonists of acetylcholine should be a valuable clue about how the compounds become attached to the receptors At least three points must be involved, Long, Luduena, Tullar, and Lands (1956) consider the likely groups in atropine to be the onium nitrogen atom, the ester carbonyl group, the tropyl hydroxyl group, and the benzene ring The receptor group binding the onium atom must be identical with that binding acetylcholine This has not been considered in detail in the discussion of the relationships between chemical structure and agonist activity (pages 207–211),some of the most valuable results, such as those of Scott shown in Table VII 13, having been obtained with antagonists Although replacement of one methyl group in the cationic head of acetylcholine by an ethyl group leads to a decline in activity, it appears to lead to an increase in affinity The drop in activity is presumably due to a big decrease in efficacy Replacement of a second methyl group by ethyl leads to a further slight increase in affinity and a marked drop in efficacy, it is remarkable how many compounds in Table VII 20 are methyldiethylammonium salts The situation at the onium nitrogen atom in tropine, however, may be different Although the tropine portion in atropine may be replaced by other amino alcohols, such as choline (or, more properly, the choline portion of acetylcholine can be replaced by tropine), the substituents on the onium nitrogen atom may not be orientated in the same way as in tropine as they are in choline Although n-butylatropinium, for instance, is feeble compared with methyl atropinium (Gyermek and Nador, 1957), a systematic study of affinity con stants and of the activity of quaternary salts of acetyltropine does not appear to have been made In quinuclidin-3-ol the arrangement of the groups in the cationic head must be, yet again, quite different An attempt to illustrate this is made in Fig VII 5, but the point is better illustrated by the examination of models It must, however, be remembered that, although the quinuclidine ring structure is rigid, the tropine structure could exist in a boat form as well as the chair form, and in acetylcholine a very great degree of flexibility is

possible There is no reason, nevertheless, to suppose that the effects on affinity of methylation must be the same in choline as they are in tropines, and they are certainly not the same in esters of quinuclidin-3-ol

The importance of the carbonyl group may be questioned, because there are many compounds with considerable activity which lack it As a polar

chair b **boat**

extended **"ring"** (Sorum 1959) c

FIG VII 5 *Possible arrangements of the groups about the onium nitrogen atom in a quinuclidin 3-ol, b tropine and c choline Note In hyoscine compounds additional attachment may be provided by the epoxide oxygen atom*

group it may contribute to some extent to the affinity, but it appears to be less important than the hydroxyl group or the benzene ring Scott (1962) has assessed the contribution of the hydroxyl group to the free energy of adsorption by comparing the affinity constants for diphenylacetic esters with those of benzilic esters The values (Table VII 22) are quite high, of the order of

TABLE VII 22

Effect of Hydroxyl Group on Free Energy of Adsorption

Figures obtained by comparison of affinity constants of diphenylacetoxyethyl and benzilyloxyethyl trialkylammonium salts

$$Ph_2CHCOOCH_2CH_2\overset{+}{N}R_3 \quad and \quad Ph_2COHCOOCH_2CH_2\overset{+}{N}R_3$$
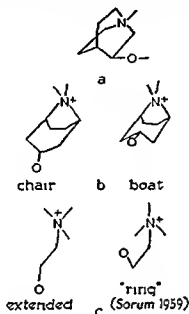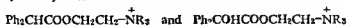
(The actual values of K are shown in Table VII 13)

$R_3 =$	$(-\Delta g, \text{ cals})$
Me₃	1,930
Me₂Et	1,830
MeEt₂	2,060
Et₃	1,750

2 kcals, and are apparently independent of the number of methyl groups attached to the onium nitrogen atom This would be consistent with the hypothesis of Long, Luduena, Tullar, and Lands (1956) that the hydroxyl

group forms a hydrogen bond with a receptor group at this point, but it cannot he the same as the receptor group which is postulated as binding the ether oxygen atom in muscarine In the latter the receptor group must contain the hydrogen otom, whereas in the former it is attached to the antagonist molecule The receptor group binding the hydroxyl group in atropine and benzilic esters, however, might possibly be the same as that which may bind the hydroxyl group in muscarine
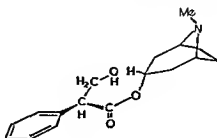
FIG VII 6
(−) *S Atropine*

The importance of the benzene ring in atropine has been known ever since the work of Pyman (1917) but must be ascribed to its size and shape rather than to any effects on the distribution of electrons within the molecule and on the activation of other groups, such as the ester link Lands and Luduena (1956) have found the *cyclohexyl* analogues are almost, though not quite, as active, and even analogues with large alkyl groups have appreciable activity Lands and Luduena describe them as 'an umbrella like mass which may form a protecting shield over the receptor surface, thereby preventing close approach of stimulating molecules such as acetylcholine' Another possibility is that this part of the molecule is held at the receptor surface by multiple Van der Waals' forces, the surface itself being flat, and consequently a benzene ring provides the best fit and strongest attachment
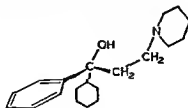
FIG VII 7
R Benzhexol

In (−) hyoscyamine the relative positions of the onium group, the hydroxyl group, and the benzene ring appear to be critical the affinity constant for the S tropyl ester, in which the groups are arranged as in Fig VII 6, being something like 100 times the affinity of the mirror image, suggesting that the free energy of adsorption differs by approximately 3 kcals If the groups in the

substituted propanolamines, such as *Benzhexol*, must be arranged similarly, it should follow that the active (−) isomer should have the R-configuration (Fig VII 7)

It may, however, be unjustifiable to compare the two types of molecule The stereospecificity of *Benzhexol* and its analogues is very odd, because it indicates that the receptor can distinguish very clearly between a benzene ring and a *cyclo*hexyl ring, yet the 1, 1-diphenyl compounds are only slightly less active than the 1-phenyl-1 *cyclo*hexyl compounds Further, the α-phenyl-α *cyclo*hexyl-β-hydroxypropionic ester of 2 diethylaminoethanol (VII 91)

Ph   CH₂OH

CO—O—CH₂CH₂NEt₂
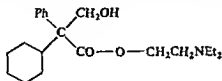
VII 91

has been prepared by Blicke and Raffelson (1952) and does not appear to be particularly active (Lands and Luduena, 1956) In the 1 1-diphenyl and 1-phenyl-1 *cyclo*hexyl compounds, moreover, the two large groups interact sterically, they cannot, for instance, both lie in the same plane It seems also likely that they may interact with the large substituents on the onium atom (which is only 3 carbon atoms away), because the degree of stereospecificity shown in Table VII 19 appears to depend upon the substituents on the onium group It is much less for the tertiary bases than for the quaternary salts, and apparently less for metho-salts than for etho salts In view of all this, the adsorption of these compounds may be quite different from that of

OH   CH₂
C
CH₂
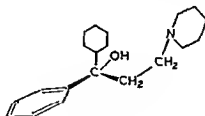
FIG VII 8

S Benzhexol

the tropyl esters The *cyclo*hexyl group may act because of its steric effects on the rest of the molecule, rather than by combining with the receptors, and the situation may be that shown in Fig VII 8 This is the mirror image of Fig VII 7, it indicates that the S compound should be active and might explain the inactivity of the α phenyl α *cyclo*hexyl β hydroxypropionic ester, VII 91 It would be very interesting to know the absolute configuration of these active (−) isomers It would also be very interesting to have more information about the activity and stereospecificity of tropyl ψ tropine

Conclusion

It is impossible to come to any definite conclusion about the structure of postganglionic cholinergic receptors The discussion in this account has been chiefly concerned with the receptors in the guinea-pig ileum, because it is on this tissue that the most precise results have been obtained Although it is possible in these circumstances to consider the interaction between pharmacodynamic groups on the drug and receptor groups, such a discussion tends to produce more queries than answers It is with antagonists that information must first be obtained, only when the effects of structure on affinity have been worked out is it possible to assess the separate effects of structure on the activity and on efficacy in agonists From the study of pairs of series of compounds, one being agonist and the other antagonist, Scott (1962) was able to show the importance of a 3-ether oxygen atom for efficacy Why this should be so and what is implied by the word efficacy at these receptors is yet another unsolved problem, so are the factors which make one drug active at the receptors in the eye when another, equally active at the receptors in the intestine, is not

VIII

Compounds Affecting Cholinesterases

Introduction – Physiological consequences of blocking cholinesterases – Uses of inhibitors of acetylcholinesterases – Physiological testing of inhibitors of cholinesterases – Biochemical testing – *Methods – Source and purity of enzyme – Degree of saturation – Assessment of inhibitory activity*
SUBSTRATES Aliphatic esters – Aromatic esters – Effects of altering the choline residue – Effect of the composition of the onium group – *Bis* onium compounds
INHIBITORS Compounds developed from eserine – Simple onium salts – More complex *bis* onium salts – Organophosphorus inhibitors of cholinesterases
Evidence for attachment at two sites in the active centre – Importance of the anionic site – Nature of groups in the esteratic site – Actions of organophosphorus compounds at the esteratic site – Reactivators – Attachment at the active centre – Relationships between ability to inhibit acetylcholinesterases and pharmacological properties – Conclusion

Introduction

In discussing the actions of acetylcholine, Dale (1914) considered the possibility that the transience of its effects might be due to its rapid destruction *in vivo* Loewi and Navratil (1926) obtained evidence that acetylcholine was, in fact, destroyed by extracts of frog heart, and Engelhart and Loewi (1930) obtained results which indicated that the destruction of acetylcholine by blood was an enzymatic process Stedman, Stedman, and Easson (1932) called the enzyme 'cholinesterase' to distinguish it from other esterases which did not hydrolyse acetylcholine so rapidly Even this distinction does not go far enough, Alles and Hawes (1940) pointed out differences between the cholinesterases of serum and of red cells, and Mendel and Rudney (1943) suggested that the non specific enzyme found in serum should be called 'pseudo-cholinesterase' Mendel, Mundell, and Rudney (1943) observed that the two types of enzyme could easily be distinguished, pseudocholinesterase, found in plasma, would hydrolyse benzoylcholine but not acetyl β methyl choline, whereas 'true' cholinesterase, found in red blood cells, in brain, and in nervous tissue, would hydrolyse acetyl-β-methylcholine but not benzoyl choline

The terms 'true' and 'pseudo' are rather misleading, particularly as it is now clear that there are not merely two different enzymes but two different types of enzymes Augustinsson and Nachmansohn (1949) and Sturge and Whittaker (1950) have used the terms acetylcholinesterases and butyryl-cholinesterases to distinguish between the two The more specific enzymes, the acetylcholinesterases, hydrolyse acetylcholine very rapidly, but fail to affect butyrylcholine, and it is enzymes of this type which destroy the acetylcholine released at nerve endings, particularly at the neuromuscular junction

Physiological Consequences of Blocking Cholinesterases

These are exemplified by the properties of the alkaloid eserine (physostigmine, VIII 20), the active principle of the Calabar Bean This substance was tested by Fraser (1864), but the first real indications of its mode of action came from the work of Fuhner (1918), who observed that it potentiated about a million-fold the action of acetylcholine on the leech muscle, i e in the presence of a concentration of eserine which itself had no effect, contracture was produced by concentrations of acetylcholine which were only one-millionth of the concentration previously required Loewi and Navratil (1926) showed that eserine stopped the destruction of acetylcholine by enzymes present in blood and suggested that it might be this which accounted for its pharmacological effects These are essentially those of acetylcholine itself, slowing of the heart, stimulation of secretions, such as salivation, contracture of plain muscle, constriction of the pupil, and ultimately death from stoppage of the respiration

Another consequence of blocking cholinesterases is to overcome the effects of substances which antagonize the actions of acetylcholine The actions of atropine at the postganglionic cholinergic receptors, for example, are antagonized by eserine and other inhibitors of acetylcholinesterases, and contrariwise many effects of these inhibitors are counteracted by atropine At the neuromuscular junction inhibition of acetylcholinesterase will lead in a reversal of the effects of blocking agents such as $(+)$-tubocurarine, although the inhibitors by themselves may lead to the accumulation of sufficient acetylcholine to cause a block in transmission

Uses of Inhibitors of Acetyl Cholinesterases

Substances which inhibit acetylcholinesterases are useful in circumstances in which transmission is disturbed because supplies of acetylcholine are either deficient or appear to be deficient because some antagonist is present. One such condition is found at the neuromuscular junction in the disease *myasthenia gravis* There are many possible causes for this, too little transmitter, too much cholinesterase, decreased sensitivity of the receptors at the end plate, the action of an antagonist of acetylcholine present in the circulation, and these are discussed later (page 280) The symptoms are muscular weakness and fatigue, e g drooping eyelids, and are often restricted to individual muscles rather than extending generally to all voluntary muscle The condition, which greatly resembles paralysis produced by $(+)$ tubocurarine, is improved by treatment with an inhibitor of acetylcholinesterase This, however, results in the accumulation of acetylcholine not only at the neuromuscular junction but also at the postganglionic cholinergic receptors with consequent slowing of the heart, excessive salivation, gastric secretion, and intestinal movement These particular side effects may require to he offset by the administration of atropine

The action of inhibitors of acetylcholinesterases at the neuromuscular junction in overcoming the neuromuscular block produced by compounds

such as (+) tubocurarine has already been mentioned and appears to depend upon similar principles to their use in the treatment of *myasthema gravis* The drawback, as in the use of any drug as an 'antidote' in a case of poisoning, is that the time course of the effects of the inhibitor of acetyl cholinesterase may be quite different from that of the neuromuscular blocking agent There may be a drastic relapse following an apparently dramatic recovery or, alternatively the effects of the inhibitor may persist long after there is any need for them In any event postganglionic cholinergic effects will be produced, which are unpleasant and may necessitate the use of atropine It may, therefore, be argued that in the event of prolonged paralysis with a neuromuscular blocking agent the most logical treatment is artificial respiration using a machine

Inhibitors of acetylcholinesterase are used in opthalmology for the treatment of glaucoma As already mentioned (page 189), cholinergic stimulation of the ciliary body may lead to improved drainage of the Canal of Schlemm, and the effects of drugs which inhibit acetylcholinesterase last much longer than those of drugs, such as pilocarpine, which act directly on the postganglionic cholinergic receptors

Inhibitors of acetylcholinesterases are a potential threat as poison gases Many such compounds, some of which are highly active, were developed during the war of 1939–45 and are known as the 'nerve gases Rather similar compounds which are more effective in inhibiting butyrylcholinesterases have also been produced and are of great value as insecticides It appears that in insects butyrylcholinesterases or closely related enzymes are more important than acetylcholinesterases

Physiological Testing of Inhibitors of Cholinesterases

As the actions of these compounds lead to the accumulation of acetylcholine, they produce effects which can be detected and compared by any of the preparations previously discussed for measuring the actions of acetylcholine and acetylcholine like compounds Stedman and Stedman (1926, 1929) for example, used the constriction of the pupil of the eye of the cat as an indication of ability to inhibit cholinesterases Salerno and Coon (1949) compared the concentrations of compounds which produced detectable changes in the spontaneously rhythmic contractions of rabbit intestine

Blaschko, Bulbring, and Chou (1949) compared the concentrations of compounds which produced comparable *degrees* of reversal of the blocking action of (+) tubocurarine on the rat diaphragm preparation The test compound and (+)-tubocurarine chloride were added simultaneously and the concentrations adjusted so that at the end of a standard time 20 per cent inhibition of the contractions was observed (Fig VIII 1) It was found that when the dose of tubocurarine chloride was plotted against the logarithm of the concentration of the test compound a straight line was obtained The logarithm of the reciprocal of that concentration which, when given with an arbitrary amount (180 μg) of (+) tubocurarine chloride resulted in 20 per

cent reduction in the size of the contractions at the end of the standard time, was termed the pD20

In all these physiological tests the response observed is not necessarily dependent upon ability to inhibit acetylcholinesterases and could be produced by substances acting like acetylcholine directly on the receptors The magnitude of the response, furthermore, depends upon the activity of the cholinesterases in the individual preparation, and this may vary considerably from one experiment to another or even during the time course of a single experiment The use of the magnitude of a physiological response, therefore, as an indication of the degree of inhibition of the enzymes is not satisfactory It would seem to be much better to assess directly the action of the compound in protecting acetylcholine from destruction by the enzyme

Tracing made by rat diaphragm preparation
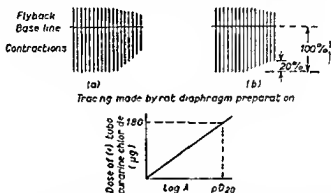
Fig VIII 1 *Physiological effects of inhibition of cholinesterases estimation of ability to reverse the neuromuscular blocking action of (+)-tubocurarine chloride*
  a *The effects of (+)-tubocurarine chloride (y µg) on the contractions of the rat diaphragm preparation*
  b *The effects of the same dose of (+)-tubocurarine chloride in the presence of the inhibitor of cholinesterases (1/A Mols)*
    The lower section shows the method of calculating pD20, if the dose 1/A Mols of inhibitor reduced the inhibition of the contractions to 20 per cent (After Bloschko, Bulbring and Chou, 1949)

Biochemical Testing

Methods

The destruction of acetylcholine can be followed by bioassay In the method described by Burn (1952) the acetylcholine is estimated on the frog rectus preparation Although this is very sensitive and substrate concentrations as low as about 5×10^{-7} M can be used, it is extremely tedious, and it is easier to follow the destruction of acetylcholine by chemical methods Neither acetylcholine nor its hydrolysis products, choline and acetic acid, can readily be estimated spectrophotometrically, although to estimate enzymic activity other substrates may be used which give, or can be made to give, light-absorbing products (Koelle and Friedenwald, 1949, Tabachnick, Mershon, Grelis, and Rubin, 1958, Main, Miles, and Braid, 1961) Most methods for

studying the hydrolysis of acetylcholine depend upon the measurement of the liberated hydrogen ions This can most simply be done by continuous electrometric titration or by measuring manometrically the carbon dioxide evolved from a bicarbonate buffer Continuous titration, using a glass electrode (Glick, 1938, Alles and Hawes, 1940), is very sensitive, and it is possible to work with concentrations as low as 10^{-6} M, but it is very time-consuming unless automatic apparatus is available The manometric method (Ammon, 1934) is less sensitive and with most equipment the lowest concentration of substrate which can be used is about 2×10^{-4} M It is, however, extremely convenient as many samples can be studied simultaneously

It is important that the pH is kept constant throughout the reaction because the activity of the enzyme varies greatly with pH The hydrogen ions must be neutralized as rapidly as they are formed, and it would not be satisfactory to use a method which involved the measurement of the amount of acid liberated after a measured time

Source and Purity of Enzyme

The effect of an inhibitor may depend greatly upon the source of the cholinesterase being studied When the anticholinesterase activity of a compound is being measured in order to obtain information about its physiological properties, it would be logical to use the same enzyme *in vitro* as is present in the tissues *in vivo* This, however, is extremely difficult to do because the amounts of enzyme present in the tissues are usually very small Moreover, it is usually necessary, especially if manometric methods are employed, to use higher concentrations of substrate (acetylcholine) than those active *in vivo* in order to obtain effects (e g volumes of carbon dioxide) big enough to be detected In experiments designed to test the ability of drugs to prevent the destruction of acetylcholine released from nerve endings, it is common to use as a source of acetylcholinesterases preparations obtained from red blood cells, from the caudate nucleus in the central nervous system, or from the electric organ of the electric eel *electrophorus electricus* In experiments with butyrylcholinesterases it is common to use serum or plasma from the animal on which the physiological tests are being performed

Neither acetylcholinesterases nor butyrylcholinesterases have as yet been obtained pure, and most experiments are performed on very crude material The richest of the sources mentioned above appears to be that from the electric eel 1 mg of this tissue will hydrolyse from 50 to 100 mg acetylcholine per hour, but purified material has been obtained, 1 mg of which is capable of splitting 75 g acetylcholine per hour The sedimentation pattern in the ultracentrifuge indicates that this is homogeneous and the molecular weight appears to be about 3 million (Nachmansohn and Wilson, 1951) Further purification has been achieved by Kewitz and Nenhoff (1960)

A survey of the properties of the cholinesterases obtained from a variety of sources has been made by Augustinsson (1948, 1949) and Nachmansohn and Wilson (1951)

Degree of Saturation

Even though the concentrations of substrate used in the *in vitro* experiments
are much higher than those used in the *in vivo* experiments, the effects of an
inhibitor *in vivo* can be assessed if the degree of saturation of the enzyme is
comparable *in vitro* with what it is *in vivo* It is also important to work *in
vitro* with an enzyme which is, as far as possible, similar to that present *in vivo*
In any experiments with inhibitors, therefore, it is necessary to begin by
studying the characteristics of the particular cholinesterase which is to be
used To obtain some idea of the degree of saturation of the enzyme in the
experimental conditions, it is simplest to plot the rate of reaction against the
substrate concentration The rate of reaction should increase with increasing
substrate concentration and at low levels of saturation of the enzyme the
reaction appears to be of the first order Alles and Hawes (1940) observed
that the reaction actually appears to be bimolecular, depending upon the
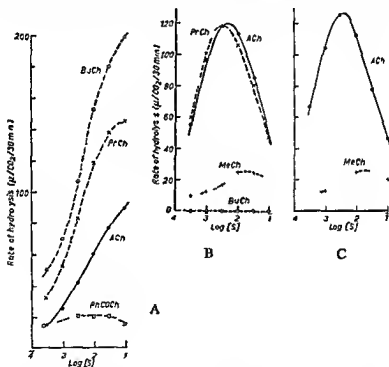
FIG VIII 2   *Effects of substrate concentration on rate of hydrolysis A, for the
butyrylcholinesterase of human serum B for crude acetylcholinesterase of the
electric eel C, for purified acetylcholinesterase (capable of splitting 20 g of acetyl
choline per hour per mg of protein)*
    *The rate of the reaction is expressed as the number of μlitres of carbon dioxide
liberated from the buffer in 30 minutes, obtained by extrapolation of the results
obtained in the early part of the experiment This is plotted against the logarithm
of the substrate concentration ACh = acetylcholine PrCH = propionylcholine
BuCh = butyrylcholine PhCOCh = benzoylcholine MeCh = (±)-acetyl β-
methylcholine (Redrawn from results of Augustinsson, 1949)*

concentration of acetylcholine and **upon** the concentration of hydroxyl ions, but in experiments at constant pH the reaction will appear to be of the first order At higher concentrations of substrate, however, the enzyme becomes saturated and the reaction appears to be of zero order The effect of an inhibitor on the rate of hydrolysis of acetylcholine in these conditions will be much less than at lower concentrations, quite a considerable degree of antagonism, as measured by the reduction in the number of acetylcholine molecules bound to the active spots in a given unit of time, may result in only a small reduction in the rate of hydrolysis

The investigation of the effect of substrate concentration on the rate of the reaction is particularly important when experiments are performed with acetylcholinesterase With enzymes of this type the curve is bell-shaped (Fig VIII 2), indicating that high concentrations of substrate inhibit the reaction With the butyrylcholinesterases, however, there is no inhibition by excess substrate The Michaelis-Menten constant for acetylcholinesterases, i e the dissociation constant of the complex formed by acetylcholine with the enzyme (page 20), appears to be between 10^{-3} and 10^{-4} M Wilson and Bergmann (1950) obtained the value $2 6 \times 10^{-4}$ M for the acetylcholinesterase of the electric eel, and this agrees closely with estimates for the acetylcholinesterases derived from other sources, such as dog brain $(2 9 \times 10^{-4}$ M) For the butyrylcholinesterases of serum the values lie between 10^{-2} and 10^{-3} M, indicating a much less stable complex

Assessment of Inhibitory Activity

In order to express the activity of inhibitors, the most logical procedure would be to test if the inhibition is competitive and, if it is, determine the inhibitor constant K_i, which is the dissociation constant of the complex formed by the inhibitor and the enzyme (page 22) For the reaction in the absence of inhibitor the rate $v = \dfrac{ke}{1 + K_s/S}$, where k is a rate constant, e the concentration of enzyme, S the concentration of substrate, and K_s the Michaelis-Menten constant for the enzyme and substrate (page 20) In the presence of inhibitor in concentration I and forming a complex whose dissociation constant is K_i, the rate

$$v' = \frac{ke}{1 + K_s/S(1 + I/K_i)}$$

(page 23) hence the ratio

$$v/v' = \frac{1 + K_s/S(1 + I/K_i)}{1 + K_s/S} = \frac{S + K_s + IK_s/K_i}{S + K_s} = 1 + \frac{IK_s}{K_i(S + K_s)}$$

For competitive antagonism, therefore, the graph of v/v' against the concentration of inhibitor should be a straight line, passing through the point $v/v' = 1$ when $I = 0$ (Fig VIII 3), and from the slope the value of K_i can be determined if S and K_s are known (Augustinsson and Nachmansohn, 1949)

I

If the effects of the inhibitor are studied at different concentrations of substrate it is possible to avoid measuring K_s directly Because

$$v/v' = \frac{S + K_s + IK_s/K_i}{S + K_s}$$

$$\frac{v'}{v' - v} = \frac{1}{v/v' - 1} = \frac{S + K_s}{IK_s/K_i} = \frac{1}{I}\left(\frac{SK_i}{K_s} + K_i\right)$$

For competitive antagonism, therefore, the graph of $I \times \dfrac{v'}{v - v}$, against substrate concentration should therefore be a straight line with a slope of K_i/K_s, and when $S = 0$ the value $I \times \dfrac{v'}{v - v}$ should equal K_i (Fig VIII 4)

This procedure was developed by Hunter and Downs (1945) for studying inhibitors of arginase
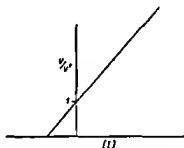
FIG VIII 3 *Graph of v/v' against inhibitor concentration, I, for a competitive antagonism, where v is the velocity of the control reaction and v the velocity in the presence of the inhibitor From the equation*

$$v/v' = 1 + \frac{IK_s}{K_i(S + K_s)}$$

$v/v' = 1$, when $I = 0$, $K_i(S + K_s) = -IK_s$, when $v/v' = 0$ and the slope of the line is $\dfrac{K_s}{K_i(S + K_s)}$

Another method has been described by Dixon (1953) and is discussed on page 23 The disadvantage of this method is that it depends upon the convergence of two lines (Fig I 9) and quite small experimental errors may lead to large changes in the estimate of K_i

If the reaction is non-competitive and of the type discussed on pages 14 and 24, in which the formation of complex by the inhibitor is independent of that formed by the substrate, the rate

$$v' = \frac{ke}{(1 + I/K_i)(1 + K_s/S)}$$

and $v/v' = 1 + I/K_i$ The graph of v/v' against I will accordingly be a straight line with a slope of $1/K_i$ so this procedure does not distinguish between competitive and non competitive inhibition, this distinction can only

he made by testing the inhibitor in different concentrations of substrate If the procedure of Hunter and Downs (1945) is followed,

$$\frac{v - v'}{v - v'} = I/K_i \text{ and } \frac{Iv'}{v - v'} = K_i,$$

which is a constant independent of S (Fig VIII 5) For this kind of inhibitor the rate of the inhibited reaction is a constant fraction $\left(\frac{1}{1 + I/K_i}\right)$ of the
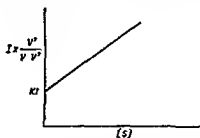
FIG VIII 4   *Graph of* $I \times \frac{v'}{v - v'}$ *against substrate concentration, S, for a competitive antagonism, where v is the velocity of the control reaction and v' the velocity in the presence of the inhibitor in a concentration I*

From the equation

$$I \times \frac{v'}{v - v'} = K_i + \frac{K_i}{K_s}(S)$$

$I \times \frac{v'}{v - v'} = K_i$ when $S = 0$, and the line has a slope of K_i/K_s,
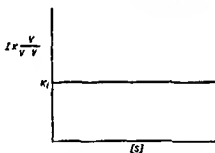
FIG VIII 5   *Graph of* $I \times \frac{v}{v - v'}$ *against substrate concentration, S, as in Fig VIII 4, but for a non competitive antagonism of the type considered on pages 14 and 24*

The value of $I \times \frac{v}{v - v'} = K_i$ and is independent of S

control reaction regardless of the concentration of substrate The equation for the rate of the inhibited reaction can be written

$$1/v' = (1 + K_s/S)(1 + I/K_i) \times 1/ke$$

and consequently the graph of $1/v'$ against $1/S$ for a particular value of I is a straight line passing through the point $1/v = 0$, $1/S = -K_s$ and differing from the graph for the reaction in the absence of inhibitor only in that it has a bigger slope $(1 + I/K_i)K_s/ke$ instead of K_s/ke Similarly, if following the

procedure of Dixon (1953), I/v' is plotted against I for particular values of S, a straight line is obtained which passses through the point $1/v = 0$, $I = -K_i$ (page 24)

For a non-competitive inhibitor, the concentration which reduces the rate of the reaction to half the control rate is a direct measure of K_i, for if $v/v' = 2$ $I/K_i = 1$ Since this is usually a small fraction, it is convenient to express this as the logarithm of $1/K_i$, which is sometimes called pI50

For a competitive inhibitor, however, the concentration which reduces the rate of the reaction to half the control rate is dependent upon the degree of saturation of the enzyme As has been shown on page 46, $1 + S/K_s = I/K_i$ and hence $-\log K_i = \log(I + S/K_s) + pI50$ For accurate estimations, therefore, it is necessary to establish the type of inhibition and the characteristics of the particular enzyme studied as well as the pI50

SUBSTRATES

Aliphatic Esters

The substrate specificity of the cholinesterases has been reviewed by Whittaker (1951) Only compounds closely related to acetylcholine are hydrolysed by acetylcholinesterases, but butyrylcholinesterases are much less specific (Table VIII 1) There is some variation in the specificity of acetylcholin esterases derived from different sources, the enzyme from *electrophorus electricus*, for instance, does not distinguish between acetylcholine and propionylcholine (Fig VIII 2), whereas the enzyme from red cells does not hydrolyse the latter as rapidly as the former (Fig VIII 6) The butyrylcholin esterases of plasma hydrolyses acetylcholine at a much slower rate than butyrylcholine (Fig VIII 2) The Michaelis–Menten constant for acetyl choline and the butyrylcholinesterases of human plasma is 10 $^{2\,5}$ (Augustins

TABLE VIII 1

Hydrolysis of Esters of Choline Rates Expressed as a Percentage of the Rate of Hydrolysis of an Equimolar Concentration of Acetylcholine

	Acetylcholinesterases		Butyrylcholinesterases		
	Electric eel	Red cells	Human plasma	Dog plasma	Horse plasma
Formylcholine	—	—	—	120	—
Propionylcholine	97	80 62	180 190	130	140
Butyrylcholine	1	8 2	270 240	—	230
n-Valerylcholine	—	1	200	—	—
Benzoylcholine	0	3	70	30	2
Carbamoylcholine	—	—	—	—	0
(±)-Acetyl β methylcholine	22	38	2	12	2
(±)-Acetyl-α methylcholine	—	—	—	—	70

Nachmansohn and Rothenburg (1945) Kahane and Levy (1936) Gl ck (1938) Sekul Holland and Breland (1962)

	Equipotent molar ratios relative to acetylcholine	
	Acetylcholinesterases	Butyrylcholinesterases
	Electric eel or red cells	Human plasma
Propionylcholine	1–3 7	0 5
Butyrylcholine	100–200	0·4
Benzoylcholine	∞	3 4
(±)-Acetyl-β-methylcholine	5–7	72
Acetylthiocholine	0·7	0 23 *
Propionylthiocholine	—	0 5
Butyrylthiocholine	—	0 4

* Figure calculated from the results of Koelle, 1950

Wurzel and Sapetka (1958) Wurzel (1959)

son, 1949) and Grelis and Tabachnik (1957) have obtained a value of $10^{-2\ 8}$ for butyrylcholine and what should be the same enzyme It would seem, then, that the difference in rate could be ascribed only to an increase in the affinity, but it is possible, at least with other substrates, that changes in the rate of hydrolysis may be due to changes in the rate constant for the breakdown of the enzyme substrate complex

An increase in the length of the aliphatic acid clearly reduces the rate of
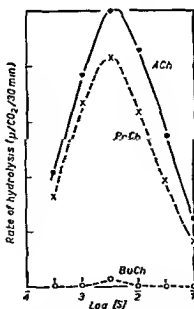
FIG VIII 6 *Effects of substrate concentration on rate of hydrolysis for the acetyl-cholinesterase of human red cells (compare with Fig VIII 2, B and C)* (Redrawn from results of Augustinsson, 1949)

hydrolysis by acetylcholinesterases, but the same is true even with butyryl-cholinesterases once the aliphatic acid is longer than *n* butyric Palmitoyl-choline, for instance, is not hydrolysed at all by the enzymes of horse serum (Glick, 1938)

The carbamic ester of choline (*Carbachol*, VIII 1) is another choline ester

$$H_2N-C\overset{O}{\underset{O-CH_2CH_2NMe_3}{\big\|}}$$
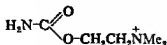
Carbachol VIII 1

which is not destroyed by cholinesterases Ammon (1935) observed that it was unaffected by the enzymes of horse serum, and Roepke (1937) showed that it would actually inhibit the hydrolysis of acetylcholine by these enzymes, although it is not a very potent inhibitor The value of K_i was $3\,8 \times 10^{-5}$ (cf the value for eserine, $2\,6 \times 10^{-9}$ in these experiments)

$$R-O-CH_2CH_2\overset{+}{N}Me_3$$

R = CH_2=CHCH$_2$CO, VIII.2
R = CH_2=CHCO, VIII 3,
R = CH_2=CMeCO VIII 4
R = CHMe=CHCO, VIII 5
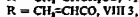
Esters of choline with unsaturated aliphatic acids have been studied by Grelis and Tabchnick (1957), Holmstedt and Whittaker (1958) and Sekul, Holland, and Breland (1962) Few of these are susceptible to hydrolysis by acetylcholinesterases the most active being vinylacetylcholine (VIII 2) and acryloylcholine (VIII 3) at about one tenth of the rate of acetylcholine for the enzyme from ox red cells, and α methylacryloylcholine (VIII 4) at about one-

TABLE VIII 2

Hydrolysis of Esters of Choline with Unsaturated Aliphatic Acids by Butyryl cholinesterases of Human Plasma

$R-COOCH_2CH_2\overset{+}{N}Me_3$		Rate expressed as percentage of rate of hydrolysis of an equimolar concentration (10^{-2} M) of acetylcholine
	R =	
Acryloylcholine	CH_2=CH–	100
Crotonylcholine	CHMe=CH–	27, 16
Vinylacetyl	CH_2=CHCH$_2$–	190
Pent 2-enoyl	CH$_3$CH=CH–	10 12
Pent-4-enoyl	CH_2=CHCH$_2$CH$_2$–	220
α Methylacryloyl	CH_2=CMe–	47
α-Methylcrotonyl	CHMe=CMe–	11
Murexine	[structure]	0
Dihydromurexine	[structure]	As rapidly as butyrylcholine – $K_i = 10^{-1.9}$

Grelis and Tabchnick (1957) Holmstedt and Whittaker (1958) Sekul, Holland, and Breland (1962)

fifth of the rate The isomeric crotonylcholine (VIII 5) is a feeble inhibitor (K_i, 1.7×10^{-4}) Many of the compounds, however, are good substrates of the butyrylcholinesterases (Table VIII 2), but the presence of an $\alpha\beta$ double bond appears to reduce the rate of hydrolysis β methylcrotonylcholine is a weak inhibitor of butyrylcholinesterase (K_i, 6.3×10^{-5})

Aromatic Esters

Aromatic esters of choline are virtually unaffected by acetylcholinesterases, but are hydrolysed moderately rapidly by butyrylcholinesterases Ormerod (1953) has studied the effects of substituents on the velocity of hydrolysis when the enzyme is saturated, i e on the rate constant for the breakdown of the enzyme–substrate complex These results are particularly interesting because they indicate the factors which may affect this rate constant and give some idea of their relative importance in determining the actual rate of hydrolysis The reaction was studied at 25°, 30°, and 37°, and the effect of temperature on the velocity constant was used to assess the activation energy

From the Arrhenius equation, $\dfrac{d \ln k}{dT} = \dfrac{E}{RT^2}$,

$$\ln k = \text{constant} - \frac{E}{RT}$$

and from values of the rate constant at different temperatures E can be calculated The results (Table VIII 3) indicate some sort of correlation

TABLE VIII 3

Hydrolysis of Substituted Benzoyl Esters of Choline Effect of Substituent on Hydrolysis and on Activation Energy (E)

Substituent	Rate constant for enzyme of horse plasma		Non-enzymic hydrolysis k_{37}	E (cals)	S
	k_{30}	k_{37}			
m-NO$_2$	2 64	4 50	0 23	13.3×10^4	$+0.71$
m-Cl	3 13	—	0 13	12 6	$+0.37$
m F	2 70	3 12	0 22	7 8	$+0.34$
p-F	1 70	2 58	0 13	11 7	$+0.06$
H	1 90	2 95	0 075	6 3	0 00
m-Me	0 64	0 81	0 10	5 1	-0.07
p-Me	0 40	0 53	0 038	7 8	-0.17
p-MeO	—	0 1 approx	0 065	—	-0.27
o Cl	1 95	2 73	0 189	—	

S is the Hammett constant log K/k_0 (page 150)

Ormerod (1953).

between the rate constant, which varied over roughly an 8 fold range, and the Hammett substituent constant, the activation energy did not change so markedly, but this depends upon changes in log k with temperature The

effects of the nature of the substituents on the spontaneous hydrolysis of these esters was much smaller than the effects on enzymatic hydrolysis From these results, therefore, it is possible to conclude that changes in the rate of enzymatic hydrolysis with structure could well be ascribable to changes in the rate constant as much as to changes in affinity, and that these effects might be due to the electronic effects of the substituents on the activation of the ester link

Effects of Altering the Choline Residue

Aliphatic esters of thiocholine are more susceptible to the action of cholinesterases than are their oxygen analogues This is indicated in Table VIII 1 and can be seen more clearly in Table VIII 4 The replacement of oxygen by

TABLE VIII 4

Hydrolysis of Esters of Choline and of Thiocholine Rate Expressed as a Per centage of the Rate of Hydrolysis of Acetylcholine (5×10^{-3} M)

	Horse serum		Ox red cells	
	pH 8 0	6 4	8 0	6 4
Acetyl thiocholine	430	160	140	83
(±)-Acetyl β-methylcholine	0	0	18	32
(±)-Acetyl β-methylthiocholine	200	—	120	—
Benzoylcholine	67	51	0	0 5
Benzoylthiocholine	40	—	0	—
Butyryl thiocholine	620	310	0	0 5
iso-Valerylthiocholine	190	—	0	—

Koelle (1950)

sulphur increases the rate of hydrolysis of acetylcholine and of acetyl β-methylcholine by both types of enzyme, acetyl β-methylthiocholine is even readily hydrolysed by butyrylcholinesterases, whereas acetyl β methylcholine is resistant With aromatic esters, however, the position appears to be reversed, benzoylthiocholine is hydrolysed less rapidly than benzoylcholine Another important observation which is shown in Table VIII 4 is that the relative rates of hydrolysis are markedly affected by pH In most experiments so far described the hydrolysis has been performed in a bicarbonate buffer usually somewhere between pH 7 and pH 8 The latter is about the optimum for the enzymes Koelle (1950) investigated the effects at pH 6 4 because at this pH the hydrolysis of acetylthiocholine can be made the basis of a histochemical test for cholinesterases Thiocholine but not acetylthiocholine will react with copper glycinate giving copper thiocholine, which can be converted to copper sulphide by treatment with ammonium sulphide By the choice of suitable substrates this method has been developed to such an extent that it is now possible to tell histochemically what type of enzyme is present and to compare roughly the amount of enzyme activity at different sites (Koelle, 1957, Holmstedt, 1957) Ravin, Tsou, and Seligman (1951) and

Ravin, Zacks, and Seligman (1953) have described a method based on the hydrolysis of β-naphthylacetate followed by coupling to give an azo-dye

The hydrolysis of the acetyl esters of α- and β-methylcholine by the butyrylcholinesterases of horse serum was studied by Glick (1938) (±)-acetyl-α-methylcholine was split at about 70 per cent of the rate of acetylcholine, but (±)-acetyl-β-methylcholine was hydrolysed at less than 2 per cent (see Table VIII 1) The hydrolysis of the latter was found to be stereospecific; the (−)-isomer was not hydrolysed, whereas the (+)-isomer was apparently hydrolysed at the same rate as the racemate Only half the racemate consists of (+) isomer so it might be thought that it should be hydrolysed at half the rate of the pure (+) form, but in these experiments the enzyme was apparently over-saturated with substrate Beckett (1962) states that the (+)-S-isomer (VIII 6) is hydrolysed by acetylcholinesterase at about half the
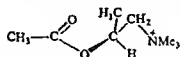
(+)-S-Acetyl β-methylcholine, VIII 6

rate of acetylcholine, whereas the (−)-R-isomer is unhydrolysed and actually inhibits the reaction The isomers of acetyl-α-methylcholine, however, are both hydrolysed at approximately the same rate as acetylcholine

Acetyl esters of various structures analogous to choline, containing hydroxyl and quaternary ammonium groups, have been studied Glick (1939) found that 2-acetoxymethyl (dimethylpyrrolidinium) (VIII 7) and (dimethylpiperidinium) (VIII 8) were hydrolysed by the butyrylcholinesterases of
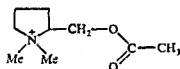
VIII 7
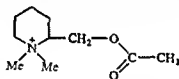
VIII 8

horse serum at 43 and 45 per cent, respectively, of the rate of acetylcholine The isomeric 3-acetoxymethyl(dimethylpiperidinium) (VIII 9) was only hydrolysed at about 5 per cent of the rate of acetylcholine and 2- and 3-acetoxyethyl or acetoxypropyl derivatives were not apparently split at all
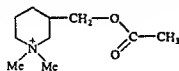
VIII 9
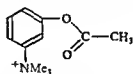
VIII 10,
o-isomer, VIII 11

Augustinsson (1949) found that *m* acetoxyphenyltrimethylammonium (VIII 10) was hydrolysed much more rapidly than the *o*-isomer (VIII 11) by the butyrylcholinesterases of human plasma and actually had a higher

affinity for the enzyme than acetylcholine itself, K_s is $10^{-3\cdot7}$ as opposed to $10^{-2\cdot5}$ for acetylcholine The rate of hydrolysis, however, at high concentrations of substrate was lower than that of acetylcholine, indicating that the rate of breakdown of the enzyme-substrate complex to yield hydrolysis products was slower than with acetylcholine

Baldridge, McCarville, and Friess (1955) and Friess and Baldridge (1956) have studied the hydrolysis of the *cis* and *trans* isomers of 2 acetoxycyclohexyl and 2 acetoxycyclopentyl trimethyl ammonium (VIII 12 and VIII 13) by

$$CH_3\text{-}CO\text{-}O \qquad {}^{+}NMe_3 \qquad CH_3CO\text{-}O \qquad {}^{+}NMe_3$$

$$n = 4 \text{ VIII 12}, \qquad\qquad n = 3 \text{ VIII 13}$$
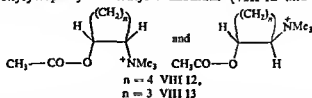
electric eel acetylcholinesterase The results (Table VIII 5) emphasize the importance of the relative positions of the ester group and the onium group because the *cis* isomers are hydrolysed faster than the *trans* With the cyclohexyl compounds the difference between the two isomers is less marked than with the cyclopentyl compounds, this could be because in the latter the distance between the two groups is more or less fixed, whereas with the cyclohexyl compounds there is some degree of flexibility It is interesting that the inhibitory activity of the products of the hydrolysis, the alcohols, is also greater with the *cis* isomers than with the *trans*

TABLE VIII 5

Hydrolysis of 2 Acetoxycyclohexyl and cycloPentyl trimethylammonium by Electric Eel Acetylcholinesterase

$$\underset{\text{CH}-\text{OOCCH}_3}{\overset{\overset{+}{\text{CH}}\cdot\text{NMe}_3}{\underset{\mid}{(\text{CH}_2)_{3\text{ or }4}}}}$$

	K_i for alcohol	Relative max velocity
Choline	$4\,5 \times 10^{-4}$	1 0
$(\pm)$-*cis*-cyclohexyl	1 1	1 14
$(\pm)$-*trans*-cyclohexyl	2 1	1 06
$(\pm)$-*cis*-cyclopentyl	0 75	1 43
$(\pm)$-*trans*-cyclopentyl	0 89	1 07

pH 7 3

Baldridge, McCarville, and Friess (1955) Friess and Baldridge (1956)

Effect of the Composition of the Onium Group

The effects on the rate of enzymatic hydrolysis of replacing methyl groups in the cationic head of acetylcholine by ethyl or acetoxyethyl are shown in Table VIII 6 With acetylcholinesterases the size of the onium group does not

TABLE VIII 6

Hydrolysis of Analogues of Acetylcholine

$CH_3COOCH_2CH_2NR_3^+$	Rate expressed as percentage of rate of hydrolysis of acetylcholine	
	Acetylcholinesterase of dog's caudate nucleus (substrate concentration 6×10^{-2} M)	Butyrylcholinesterase of horse serum (substrate concentration 10^{-2} M)
$R_3 =$		
Me_2Et	110	72
$MeEt_2$	96	49
Et_3	94	26
$Me_2(CH_3CH_2O-OC-CH_3)$	106	84
$Me(CH_2CH_2O-OC-CH_3)_2$	22	8

Holton and Ing (1949)

appear to be particularly important, but with butyrylcholinesterases an increase in the size of the onium group considerably reduces the rate of hydrolysis

Roepke and Welch (1936), using haemolysed human blood as a source of cholinesterases, could not detect any difference in the rates of hydrolysis of acetylcholine, acetylphosphocholine (VIII 14) and acetylarsenocholine (VIII 15) Using horse serum, Roepke (1937) found the Michaelis–Menten constant to be $8\,0 \times 10^{-5}$ for acetylcholine and $8\,5 \times 10^{-5}$ for acetylarseno choline

$$CH_3-C \Big\langle {}^{O}_{O-CH_2CH_2-X}$$
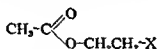
$$X = \overset{+}{P}Me_3 \quad VIII\ 14$$
$$X = \overset{+}{A}sMe_3 \quad VIII\ 15$$
$$X = \overset{+}{S}Me_2 \quad VIII\ 16$$
$$X = CMe_3 \quad VIII\ 17$$

The sulphonium analogue of acetylcholine, (acetoxyethyl)-dimethylsulphonium (VIII 16, Prelog, Juhasz, Rezek, and Stern, 1942), does not appear to be hydrolysed quite as rapidly as acetylcholine by the enzymes of horse serum

The carbon analogue of acetylcholine, 3 3-dimethylbutylacetate (VIII 17, Adams, 1949, Adams and Whittaker, 1949), in which the positively charged quaternary nitrogen atom is replaced by an uncharged quaternary carbon atom, is hydrolysed by the acetylcholinesterases of human red cells at about 60 per cent of the rate of hydrolysis of acetylcholine, in concentrations of substrate which should give maximal rates of hydrolysis This rate is actually 1 8 times as fast as the rate of hydrolysis of acetyl β methyl choline by this nzyme, this latter substance being hydrolysed at about 33 per cent of the

rate of hydrolysis of acetylcholine (cf Table VIII 4) With the butyryl-cholinesterase of human plasma, however, 3·3-dimethylbutylacetate is hydrolysed only at about 35 per cent of the rate of hydrolysis of acetylcholine

Bis-onium Componnds

A series of polymethylene *bis* acetoxyethyldimethylammonium salts (VIII 18) was studied by Barlow (1955), and it was found that the rate of hydrolysis varied greatly with the length of the polymethylene chain (Table VIII 7)

$$R-O-CH_2CH_2\overset{+}{\underset{Me}{\overset{Me}{N}}}-(CH_2)_n-\overset{+}{\underset{Me}{\overset{Me}{N}}}CH_2CH_2-O-R$$

R = CH₃CO, VIII 18,
R = H, VIII 19
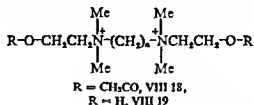
With the acetylcholinesterase of dog's caudate nucleus there was a sharp maximum in the rate of hydrolysis at the octamethylene compound, which was hydrolysed at about 60 per cent of the rate of hydrolysis of acetylcholine, though it must be remembered that this compound contains two ester groups

TABLE VIII.7

Hydrolysis of Polymethylene bis-Acetoxyethyldimethylammonium Salts

$CH_3COOCH_2CH_2\overset{+}{N}Me_2$ $\underset{(CH_2)_n}{}$ $CH_3COOCH_2CH\text{-}\overset{+}{N}Me_2$	Rate of hydrolysis as percentage of rate of hydrolysis of acetylcholine (substrate concentration, 10^{-3} M)	
	Acetylcholinesterase of dogs' caudate nucleus	Butyrylcholinesterase of horse serum
n =		
4	0	25
5	4	72
6	8	67
7	30	88
8	61	77
9	36	84
10	29	71
11	12	76
12	9	72

Barlow (1955)

With the butyrylcholinesterase of horse serum the effect of chain length on rate of hydrolysis was less marked, all the compounds with a polymethylene chain of more than 4 carbon atoms were split at rates of between 70 and 90 per cent of that of acetylcholine, but there is the suggestion that the odd-numbered members were split more rapidly than the even numbered Although these compounds were substrates of the cholinesterases, they themselves in-

hibited the hydrolysis of acetylcholine in experiments in which both substrates were present The polymethylene bishydroxyethyldimethyl ammonium compounds (VIII 19) produced by the hydrolysis of these acetoxy compounds also inhibited the hydrolysis, with both types of enzyme, affinity (i e inhibitory activity) appeared to increase with chain length, being greatest in the dodecamethylene compounds, the highest homologue studied

Although it is possible by studying the substrate specificity of the cholinesterases to obtain some picture of the structure of the active spots on the enzymes and of the processes occurring at them (see, for instance, Adams and Whittaker, 1950), much additional information, particularly about the structure of the active spots, can be obtained from considering the chemical structure and actions of substances which are only inhibitors of the enzymes These will therefore be discussed next

INHIBITORS

Compounds developed from Eserine

Eserine (VIII 20) is a tricyclic structure containing a urethane group To try to discover what part of the molecule might be associated with physiological activity, Stedman and Stedman (1926, 1929) made three series of urethanes,
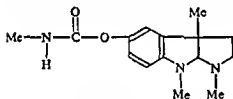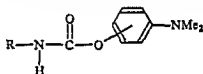
Eserine VIII.20   VIII.21
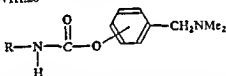
VIII 22

derivatives of dimethylaminophenols (VIII 21), of (dimethylaminomethyl)-phenols (VIII 22), and of (α-dimethylaminoethyl)phenols (VIII 23) The compounds were tested for their ability to produce constriction of the pupil in cats' eyes and to inhibit the hydrolysis of methylbutyrate and of tributyrin
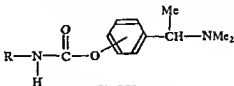
VIII.23
m- Compound R = Me,
Miotine, VIII 24

by an esterase obtained from pig liver The most effective compounds were esters of N-methylcarbamic acid, the compound *Miotine* (VIII 24) being particularly potent The corresponding phenylcarbamic esters were inactive The

effects of the orientation of the substituents and of quaternization were quite different in the three series (Table VIII 8) The increase in activity brought about by quaternization in some series is not observed with eserine itself Eserine methiodide is slightly less active than eserine itself both in tests *in vivo* (Aeschlimann and Reinert, 1931) and *in vitro* as an inhibitor of the

TABLE VIII 8
Miotic Activity of Methylcarbamic Esters on Cats' Eyes

	Base	Quaternary salt	Effect of quaternization
MeNHCOO—⟨ ⟩—NMe₂	o > p > m	m > o and p	m increased, o and p decreased
MeNHCOO—⟨ ⟩—CH₂NMe₂	o > p > m	o > m, p inactive	o increased, m decreased, p abolished
MeNHCOO—⟨ ⟩—CHMeNMe₂	m > o = p		

Stedman and Stedman (1926, 1929)

butyrylcholinesterases of horse serum (Schweitzer, Stedman, and Wright, 1939)

Unfortunately, the enzymes studied in this work do not resemble the acetylcholinesterases present at the synapses where acetylcholine is transmitter, and there was no strict correlation between physiological effects and the ability to inhibit the enzymes The most active inhibitor, the methylcarbamic ester of *m* hydroxyphenyltrimethylammonium (VIII 25), was not as

VIII 25
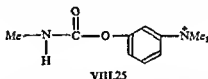
active as *Miotine* in producing constriction of the pupil White and Stedman (1937) tested the two optical isomers of *Miotine* and found the (—)-isomer to be more toxic, but as inhibitors of the cholinesterases of serum the (—) isomer was not always more active than the (+) isomer, with serum from some species the latter was the more active, but in none of the tests was there a high degree of stereospecificity The isomers differed in activity at the most only by a factor of about 3

Aeschlimann and Reinert (1931) examined dialkylcarbamic esters, which, in contrast to eserine and *Miotine*, are stable in aqueous solution These were tested on cats' eyes and on isolated rabbit intestine Of the *m* compounds the most active was Neostigmine (*Prostigmine*, VIII 26) The corresponding tertiary base (VIII 27) was only feebly active, about 100 to 200 times as much was needed in order to obtain miosis, and between 25 and 100 times as much of the diethylamino analogue (VIII 28) Other examples indicated that when

the phenols contained a quaternary group, the esters of dimethylcarbamic acid were more active than those of monomethylcarbamic acid, e.g. the methiodide of the dimethylcarbamic ester of 8-hydroxyquinoline (VIII 29) was more active than the methiodide of the corresponding monomethyl-carbamic ester (VIII 30)
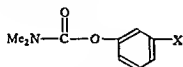
X = $\overset{+}{N}Me_3$, Neostigmine, VIII.26,
X = NMe₂, VIII.27,
X = NEt₂, VIII.28
X = $\overset{+}{N}Et_2Me$, F 3393, VIII 34
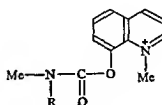
R = Me, VIII 29,
R = H, VIII 30

The monophenylcarbamic ester analogous to Neostigmine (VIII 31) was inactive, but the phenylmethylcarbamic ester (VIII 32) had feeble activity (Aeschlimann and Stempel, 1946) This was increased about 100-fold by substitution of a p-chloro group, and to a less extent (about 20-fold) by a p-methyl group, in the phenylmethylcarbamyl part of the latter, but was decreased by substitution of other groups such as o- and m-chloro or methyl The p-chlorophenylmethylcarbamic ester (VIII 33) appeared to be more active than Neostigmine, the equipotent molar ratio on rabbit ileum and as an inhibitor of the hydrolysis of acetylcholine (concentration not stated) by the acetylcholinesterase of electric eel was around 0 2
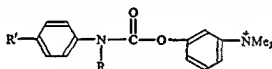
R = H, R = H, VIII 31,
R = Me, R = H, VIII 32,
R = Me, R' = Cl, VIII 33

The abilities of some of these compounds to inhibit cholinesterases are shown in Table VIII 9 This also shows the effects on activity of replacing methyl groups in Prostigmine by ethyl groups, the high activity *in vivo* of the methyldiethylammonium compound, F 3393 (VIII 34), had been noted by Schweitzer, Stedman, and Wright (1939) The values of $-\log K_I$ in these experiments appear to be 8 4 for eserine and 8 7 for Neostigmine, but these may not be very accurate because of the high degree of saturation of the enzyme ($s = 20 \times K_a$) Augustinsson and Nachmansohn (1949), using the method described on page 247, obtained values of 7 2 for eserine and 6 8 for Neostigmine with the acetylcholinesterase of the electric eel Wilson (1955) obtained a value of 7 0 for Neostigmine and electric eel acetylcholinesterase at 25° Todrick (1964), using the cholinesterases of rat brain, obtained values of 6 5 for the pI₅₀ of eserine and 6 6 for Neostigmine with 12 mM acetylcholine as substrate at 38° For the butyrylcholinesterases of horse serum, Schweitzer, Stedman, and Wright (1939) found eserine to be a slightly more powerful

TABLE VIII 9

Effects of Eserine, Miotine, Neostigmine and Related Compounds on Cholinesterases

		pIso AcetylChE of dogs caudate nucleus	pIso ButyrylChE of horse serum	pD20
Me₂N COO–⟨ring⟩–NMe₂		7 4	7 2	7 60
–NMe₂Et	3392	8 0	7 3	8 19
–NMeEt₂	3393	8 2	8 0	8 57
–NEt₃	5208	7 2	7 4	6 59
Me₂NH COO–⟨ring⟩–CHNMe₃ (Me)	Miotine	7 2	6 4	7 85
MeNH–COO–⟨ring structure⟩	Eserine	7 1	7 7	7 21
⟨quinoline structure⟩	38	7 1	7 6	6 23
Cl–⟨ring⟩–NCOO–⟨ring⟩–NMe₂ (Me)	Nu 1250	7 4	7 9	7 26
Me–⟨ring⟩–NCOO–⟨ring⟩–NMe₂ (Me)	Nu 1197	6 9	7 1	7 31
Me₂NCOO–⟨ring⟩	Nu 683	6 2	8 5	5 44
Me₂NCOO–⟨pyridine⟩ (Me NCH₃)	5130 Pyridostigmine	6 4	5 8	5 26
Me₂NCOO–⟨ring⟩–NMe₂	5220/5	4 5	4 4	4 77

The substrate for the experiments with acetylcholinesterases was acetylcholine 6×10^{-3} M if it is assumed that K_s for this enzyme is 3×10^{-4} M (page 247) values of $-\log K_i$ can be obtained by adding log 21, i e 1 32, to the values of pI₅₀ (page 250) The substrate in the experiments with butyrylcholinesterases was benzoylcholine, 6×10^{-3} M The value of pD₂₀ is an estimate of ability to antagonize the effects of $(+)$-tubocurarine on the rat diaphragm preparation (page 243) Temperature 37° C.

Blaschko, Bulbring, and Chou (1949)

inhibitor than Neostigmine, but eserine methiodide slightly less powerful The monomethylcarbamyl analogue of Neostigmine (VIII 25) was more active, being comparable with the methylethylammonium compound, F 3393

The compound (m hydroxyphenyl)ethyldimethylammonium (*Edrophonium, Tensilon*, VIII 35), which lacks the dimethylcarbamyl group, is a particularly interesting substance because it reveals some of the weaknesses in the experi-

mental methods The ability of this compound to reverse the neuromuscular blocking action of (+)-tubocurarine has already been mentioned (page 113) but the earliest experiments (Randall, 1950, Hubbiger, 1952) indicated that it was only a feeble anticholinesterase, about one hundredth as potent as Neostigmine Smith, Cohen, Pelikan, and Unna (1952) however, obtained results which indicated that it was as much as one-quarter as active as Neostigmine Wilson (1955) subsequently showed that for the acetylcholinesterase of electric eel at 20°, K_i was 3×10^{-7} for *Edrophonium* as opposed to 10^{-7} for Neostigmine The rate of formation (and of dissociation) of the complex with Edrophonium,

$$HO - \underset{}{\bigcirc} - \overset{+}{N}Me_2Et$$

Edrophonium VIII 35

however, was very much greater than that for Neostigmine If the experiments were performed in equilibrium conditions this should not affect the results, but if the association and dissociation of the Neostigmine does not have time to reach equilibrium, it may appear very much more powerful than *Edrophonium*, in these circumstances it is, in fact, behaving as a non-competitive 'irreversible' inhibitor
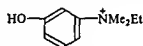
Simple Onium Salts

Alkyltrimethylammonium salts are only feeble inhibitors of cholinesterases and polymethylene *bis* trimethylammonium salts are only slightly more active Affinity for the enzymes, however, increases with chain length, and Bergmann and Segal (1954), using the acetylcholinesterase of electric eel, observed a regular increase in $-\log K_i$, consistent with the idea that each methylene group in the alkyltrimethylammonium compounds contributes about 300 cals to the stability of the complex With the *bis* trimethylammonium salts the increase was not so regular, being greater in the range of 8 to 12 methylene groups, the octadecamethylene compound, however, was found by Paton and Zaimis (1949) to he less active than the dodecamethylene The pI$_{50}$ for Decamethonium for electric eel cholinesterase at 23°, with a substrate concentration of 4 mM acetylcholine, was 4 6 (Nachmansohn and Wilson 1951) For decamethylene *bis* hydroxyethyldimethylammonium (VIII 36) and the acetylcholinesterase of dogs' caudate nucleus at 37° and a

$$HOCH_2CH_2\overset{+}{\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{N}}} (CH_2)_{10} \overset{+}{\underset{\underset{Me}{|}}{\overset{\overset{Me}{|}}{N}}} CH_2CH_2OH$$
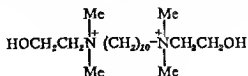
VIII 36

substrate concentration of 1 mM acetylcholine the pI$_{50}$ was 4 9, and for the dodecamethylene compound it was 6 0 (Barlow, 1955) In the same conditions much higher activity was observed in polymethylene *bis*-quinnlinium salts (Barlow and Himms 1955), the pI$_{50}$ for the decamethylene compound (VIII 37) was 7 2, comparable with that of Neostigmine Higher members of this series were prepared and tested as antifungal agents by Collier, Potter, and

Taylor (1955), but their effects on cholinesterases do not appear to have been studied Decamethylene *bis* pyridinium (VIII 38) is much less active (Hazard *et al*, 1952, Lane, 1953)

$$R-(CH_2)_{10}-R$$

$$R = \text{VIII 37,}$$

$$R = \text{, VIII 38}$$
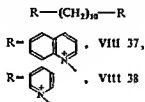
More Complex *bis*-onium Salts

Considerable activity is also found in more complex *bis*-onium salts, and compounds of the type (VIII 39) studied by Austin and Berry (1953) and by Fulton and Mogey (1954) are particularly interesting because they have a much higher affinity for acetylcholinesterase than for butyrylcholinesterase The compounds 297 C 50 (284 C 51, VIII 40) and 298 C 50 (VIII 41) had a pI$_{50}$ for the

$$R-\boxed{}-CH_2CH_2-X-CH_2CH_2-\boxed{}-R$$

X = CO, CHOH, CH$_2$ VIII 39,

X = CO, R = CH$_2$CHCH$_2$NMe$_3$—, 297 C 50, VIII 40,

X = CO, R = n—PrNMe$_2$—, 298 C 50, VIII 41
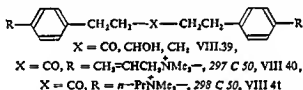
acetylcholinesterases of rat brain at 37°, with a substrate concentration of 12 mM acetylcholine, of 7 8, compared with 6 6 for Neostigmine and 6 5 for eserine The value for the enzymes of horse serum with 8 mM benzoylcholine as substrate was less than 3 0 compared with 7 6 for Neostigmine and 8 0 for eserine

Jacob (1955) observed that the compound 3318 CT (VIII 42) was an ex

3318 CT VIII 42
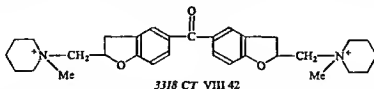
tremely active and selective inhibitor of acetylcholinesterases The value of K$_i$ for the acetylcholinesterase of dog red cells was 1 2 × 10^{-9}, whereas for the butyrylcholinesterases of dog plasma it was 3 × 10^{-6}

Long chain polymethylene *bis* carbamyl esters of choline, whose neuro-muscular blocking activity has already been discussed (page 116), also possess considerable ability to inhibit cholinesterases, which affects their behaviour at the neuromuscular junction Klupp, Kraupp, Stormann, and Stumpf (1953) found activity to be maximal in the ester of octamethylene *bis*-carbamic acid, which was comparable with eserine (Table VIII 10) Analogous esters of *m* hydroxyphenyltrimethylammonium were even more active

TABLE VIII 10

Inhibition of Acetylcholinesterases by Polymethylene bis Carbamic Esters

$$R-O-\overset{\overset{\displaystyle O}{\|}}{C}-NR'-(CH_2)_n-NR'-\overset{\overset{\displaystyle O}{\|}}{C}-OR$$

	pI$_{50}$		
	R = CH$_2$CH$_2$NMe$_3^+$	R = $\overset{Me_2N}{\diagdown}$	R = $\overset{Me_2N}{\diagdown}$
	R = H	R = H	R' = Me
	(a)	(b)	(b)
n =			
2	3 8	—	—
4	4 2	5 7	—
6	4 9	8 5	8 4
8	6 5	9 0	9 4
10	5 7	—	10 0
Eserine	6 6	—	—
Carbamoylcholine	2 5	—	—
Neostigmine	—	8 0	—

(a) Enzymes of dogs' caudate nucleus, substrate 15 mM acetylcholine.
(b) Enzymes of dogs' red cells, substrate 80 mM acetylcholine

Klupp, Kraupp, Stormann, and Stumpf (1953), Kraupp, Stumpf, Herzfeld, and Pillat (1955)

(Klupp, Kraupp, Schwarzacher, and Stumpf, 1955, Kraupp, Stumpf, Herzfeld, and Pillat, 1955) These compounds also inhibited the butyrylcholinesterases of horse serum, but not to the same extent

Funke, Depierre, and Krucker (1952) reported very high activity indeed in the somewhat similar compounds *3152 CT* (VIII 43) and *3113 CT* (VIII 44),

$$R-O-CH_2-CH_2-CH_2-O-R'$$

R = R' = $\overset{+}{N}Me_2$-⟨⟩ , *2842 CT*. VIII 45.

R = $\overset{+}{N}Me_2$-⟨⟩ , R' = Me$_2$N-C-O-⟨⟩-$\overset{+}{N}Me_2$, *3152 CT* 43.

R = R' = Me$_2$N-C-O-⟨⟩-$\overset{+}{N}Me_2$, *3113 CT*. VIII 44
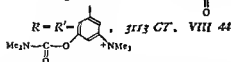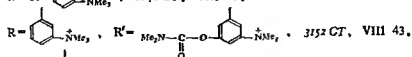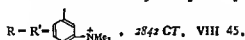
the former was reported to produce 50 per cent inhibition of the acetylcholinesterase of dog red cells in concentrations of the order of 10^{-16} M, but Levin and Jandorf (1955) obtained pI$_{50}$ values of 8 8 for *3152 CT*, 8 4 for *3113 CT*, 6 1 for the compound without carbamyl group (*2842 CT*, VIII 45), and 7 15 for Neostigmine, using the acetylcholinesterase of human red cells

at 25° and a substrate concentration of 7 4 mM acetylcholine Levin and Jandorf observed that it was much more difficult to wash these compounds off the enzyme preparations than it was to remove Neostigmine Complete removal could, however, be achieved by persistent washing

Lands, Karczmar, Howard and Arnold (1955) and Lands, Hoppe, Arnold, and Kirchner (1958) have described the properties of a series of *bis* onium salts, of which the most interesting are *Ambenonium* (*Mytelase* Win 8077, VIII 46) and its methoxy analogue *Methoxyambenonium* (*Win 8078*, VIII 47)
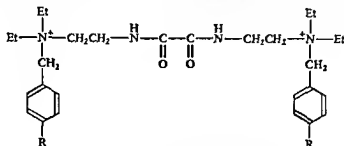
R = Cl, *Ambenonium*, VIII 46,
R = MeO *Methoxyambenonium* VIII 47

Ambenonium has considerable anticholinesterase activity, Arnold, Soria, and Kirchner (1954) estimated it to be about six times as potent as Neostigmine in inhibiting the hydrolysis of acetylcholine by the enzymes of red cells The methoxy analogue has much less anticholinesterase activity, Karczmar (1957) estimated it to be one fortieth as potent as *Ambenonium* and Koelle (1957) obtained pI$_{50}$ values of 7 6 for *Ambenonium* and 5 6 for *Methoxyambenonium* for inhibition of the hydrolysis of 30 mM acetyl β-methylcholine by the esterases of cat brain at 37° Blaber (1960) obtained values of 8 2 and 6 0, respectively, for inhibition of the hydrolysis of 14 mM acetylcholine by enzymes obtained from the cat tibialis muscle Notwithstanding this difference in anticholinesterase activities, both compounds had marked effects in facilitating transmission at the neuromuscular junction, comparable degrees of reversal of neuromuscular block produced by (+) tubocurarine were obtained with doses of *Methoxyambenonium* which were only four times those of *Ambenonium* Both compounds sensitised the frog rectus muscle to acetylcholine in concentrations which would not appear likely to affect the enzyme, the concentration of the methoxy compound was only three times that of *Ambenonium* *Methoxyambenonium* was also peculiar in that it reversed neuromuscular block produced by Decamethonium as well as that produced by (+) tubocurarine Histological work by Koelle (1957) however, has shown that at the neuromuscular junction and also in ganglia, these compounds inhibit cholinesterases in concentrations which, *in vitro*, would not be expected to produce any inhibition i e they appear to be selectively taken up at the sites where transmission occurs Moreover, although the compounds potentiate the action of acetylcholine on the frog rectus muscle, they do not potentiate the effects of substances, such as *n* pentyltrimethylammonium,

which are not susceptible to hydrolysis by cholinesterases Accordingly it may
be possible to interpret events at the neuromuscular junction without in-
voking a direct 'anticurare' or other action by the drugs, particularly as the
compounds themselves have neuromuscular blocking properties which re-
semble those of (+)-tubocurarine, these could account for the reversal of the
neuromuscular block produced by Decamethonium (Blaber, 1960) A similar
combination of ability to inhibit acetylcholinesterase and to produce, in
higher concentrations, neuromuscular block resembling that of (+) tubocur-
arine has been observed in decamethylene*bis* hydroxyethyldimethylammonium
(VIII 36) and this compound, likewise, appears to antagonize in certain con-
ditions both (+)-tubocurarine and Decamethonium (Tella, 1960)

Organophosphorus Inhibitors of Cholinesterases

During the war many highly toxic organophosphorus compounds were de-
veloped for use as poison gases, sometimes called the 'nerve gases' These can
be grouped together (Ford-Moore, 1953) as having the general structure

$$
\begin{array}{c}
R \\
\diagdown \\
P \\
\diagup \diagdown \\
R' \quad X
\end{array}
\quad O
$$

where R and R' are alkyl, alkoxy, or substituted amino groups and X is a
group, such as halogen or phosphate, which is capable of being lost as a
negative ion Substances of this type are, accordingly, derivatives of phos-
phinic, phosphonic, or phosphoric acids, depending on the nature of the
substituents R and R'

One of the first compounds to be examined, for instance, was dimethyl-
phosphorofluoridate (VIII 48), but this was much less toxic than the di*iso*-
propyl ester (*DFP*, VIII 49, Kilby and Kilby, 1947) One of the most striking
effects of exposure to sub-lethal amounts of *DFP* is the development of con-
striction of the pupil of the eye, and the effects may persist for weeks Sub-

R = MeO, VIII 48,
R = isoPrO, *DFP*, VIII 49

TEPP, VIII 50
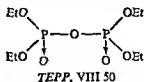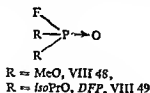
stances of this type were shown to inhibit cholinesterases (Adrian, Feldberg,
and Kilby, 1947), and it seemed very likely that this accounted for the phar-
macological properties of the compounds Although *DFP* inhibited both
acetyl- and butyryl-cholinesterases, it affected the latter more than the former
(Hawkins and Mendel, 1947, Table VIII 11)

Similar results were obtained with phosphate esters, such as tetraethyl-
pyrophosphate (*TEPP*, VIII 50) and hexaethyltetraphosphate, and a com-
parison of the concentrations of the compounds producing a detectable in-
crease in the spontaneous movements of isolated rabbit intestine is shown in
Table VIII 12

TABLE VIII 11
Inhibition of Cholinesterases by DFP

DFP concentration (Molar)	Percentage inhibition of hydrolysis Enzymes of dog red cells Substrate	
	1 2 mM Acetylcholine	30 mM Mecholyl
10^{-7}	4	3
5×10^{-7}	25	26

DFP concentration (Molar)	Enzymes of dog pancreas Substrate	
	60 mM Acetylcholine	6 mM Benzoylcholine
10^{-8}	87	86
5×10^{-8}	100	100

Mecholyl = (±)-acetyl β-methylcholine
Hawkins and Mendel (1947)

Although the concentrations of these organophosphorus compounds which produce an effect are very similar to those of substances such as eserine or Neostigmine, the effects are much more persistent and are not reversed by washing the preparation Their action is often described as 'irreversible', but this is slightly misleading Augustinsson and Nachmansohn (1949) observed that the inhibition of cholinesterases by *DFP* and *TEPP* was not competitive, the reaction can be regarded as being roughly a non-competitive one such as has been described on pages 14 This only implies, however, that the stability of the enzyme-substrate complex is so great that it is unlikely to be broken up *in vivo* Albert (1960) quotes about 10 Kcal as the greatest strength of a

TABLE VIII 12
Effects of Inhibitors of Cholinesterases on the Rhythm of Spontaneously Contracting Rabbit Intestine

	Minimum concentration producing increase	Wash effect
TEPP	3 5 × 10^{-8}	—
Eserine	3 5 × 10^{-8}	+
Neostigmine	4 3 × 10^{-8}	+
HETP	9 8 × 10^{-8}	—
DFP	5 4 × 10^{-7}	—

— Washing the preparation after administration of the drug did not stop the pharmacological response
+ Washing did stop the response
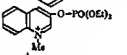
Salerno and Coon (1949)

bond which can be broken up at body temperature Compounds have been developed, however, which form a more stable complex with these organo phosphorus compounds than does the enzyme, and can thus re activate the 'irreversibly blocked enzyme These reactivators are discussed on page 276 The process of inhibition of enzymes by organophosphorus compounds, such as *DFP* or *TEPP*, accordingly consists of two stages, adsorption at the active centre followed by reaction with it From the arguments set out on page 250,

TABLE VIII 13

Inhibition of Cholinesterases by Organophosphorus Compounds

	pI_{50}	
	Acetyl cholinesterases	Butyryl cholinesterases
	(a)	(b)
DFP	6 1	7 8
TEPP	7 8	7 9
Dimethyl(p-nitrophenyl)phosphate	7 4	5 2
Diethyl(p-nitrophenyl)phosphate (*Paraoxon*)	7 8	7 6
Di isopropyl(p-nitrophenyl)phosphate	6 5	7 1
NN -di isopropylphosphorodiamidic fluoride (*Mipafox*)	4 3	6 8
	(c)	(d)
Phosphostigmine Me₂N—⟨ ⟩—O·PO(OEt)₂	7 1	8 9
Ro3–0422	9 5	8 9
MePO(F)—OCH₂CH₂NMe₃	10·0	8 4
—OCH₂CH₂CH₂NMe₃	11·0	8 4
—OCHMeCH₂NMe₃	8 4	8 4

(a) Enzymes of rat brain substrate 30 mм acetyl-β-methylcholine
(b) Enzymes of cat heart substrate 30 mм butyryl-choline
(c) Enzymes of human red cells substrate 30 mм acetyl-β-methylcholine
(d) Enzymes of human plasma substrate 10 mм benzoylcholine

Davison (1955) Burgen and Hobbiger (1951) Hobbiger (1954) Tammelin (1958)

the affinity of the compound for the active centre will be approximately indi cated by the value of pI_{50}, which should be independent of the substrate con centration The values of pI_{50} for some of the compounds are shown in Table VIII 13, and indicate that the affinities of these compounds for the active centre are not very different from those of the 'reversible inhibitors such as eserine or Neostigmine Attempts have been made to improve affinity by in corporating phosphorylating groups into molecules such as Neostigmine, which are potent reversible inhibitors of cholinesterases The resultant

compounds, e g *Phosphostigmine* (VIII 51) and *Ro3-0422* (VIII 52) appear to be highly active (Burgen and Hobbiger, 1951, Hobbiger, 1954), as are also derivatives of choline itself (Tammelin, 1958)
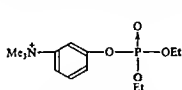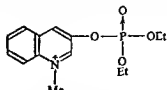
Phosphostigmine, VIII 51   *Ro3 O422, VIII 52*

Some of the compounds (e g *Mipafox* VIII 53) have been used as insecticides but, with these, ability to block butyrylcholinesterases appears to be more important than ability to block acetylcholinesterases Possibly the latter type of enzyme is less important in insects than in man Factors such as stability and ability, or inability, to penetrate the tissues of plants and insects
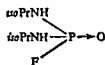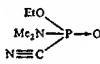
Mipafox, VIII 53   *Tabun, VIII 54*   *Sarin, VIII.55*

also greatly influence the usefulness of a particular compound These factors are also important with 'nerve gases', *Tabun* (VIII 54) and *Sarin* (VIII 55) possessing stability as well as high inhibitory activity (the pI_{50} values being 7 8 and 8 5, respectively, for the enzymes of human red blood cells, Grob and Harvey, 1958)

Evidence for Attachment at Two Sites

Much information about the nature of the active centre has been obtained by considering the chemical structure of substances which have affinity for the enzymes (either as substrates or inhibitors) and the effects of pH on the activity of the enzymes and of these substances Because of the shape of the graph of substrate concentration against rate of reaction for the enzymes of red cells, and its difference from the graph for the enzymes of plasma, Zeller and Bissegger (1943) suggested that the substrate might be attached at two *points in the active centre of the former enzyme as opposed to one in the latter* The inhibition of the reaction by high concentrations of substrate might arise from crowding of the enzyme surface leading to adsorption of the substrate at only one site, whereas only adsorption at both could lead to hydrolysis (Fig VIII 7) The most likely groups in acetylcholine which could be bound to the enzyme surface are the cationic head and the ester group (Adams and Whittaker, 1950, Wilson and Bergmann, 1950, Bergmann, Wilson, and Nachmansohn, 1950, Wilson, Bergmann, and Nachmansohn, 1950) The sites on the enzyme which may bind these have been called 'anionic' and 'esteratic' respectively (Wilson and Bergmann, 1950) From the relative

affinities of acetylcholine and 3 3 dimethylbutylacetate (page 257) for the enzymes of red cells and of plasma, Adams and Whittaker (1950) have concluded that the anionic site is, in fact, much less important in the hydrolysis of esters by the butyrylcholinesterases of plasma
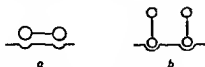
FIG VIII 7 *Inhibition of the reaction by high concentrations of substrate If the substrate has to be attached at two points, as in a, for hydrolysis, high concentrations of substrate may lead to the situation shown in b, in which hydrolysis cannot take place even though the active spot is occupied*

Importance of the Anionic Site

Although the anionic group appears to be important in acetylcholinesterases, it seems to be principally an anchoring site, the esteratic site being that involved in fission The inhibitory actions of onium salts, for instance, can probably be ascribed solely to affinity for the anionic site, coupled with Van der Waals' binding to the enzyme surface in a non specific manner The polymethylene *bis* quinolinium salts may owe their high inhibitory activity in addition to the ability of the large quinoline ring to mask the esteratic site

For substrates, however, the anionic site does not appear to be so important, probably because affinity is not the only factor influencing the rate of hydrolysis The compound 3 3 dimethylbutylacetate is, after all, hydrolysed quite rapidly by acetylcholinesterases (at about 60 per cent of the rate of acetylcholine itself) Wilson and Bergmann (1950) obtained similar results in a study of the effects of pH on the relative rates of hydrolysis of acetylcholine and β dimethylamino-ethylacetate (VIII 56) by the acetylcholinesterases of electric

$$CH_3-C\begin{smallmatrix}O\\\\O-CH_2-CH_2-NMe_2\end{smallmatrix}$$

VIII 56
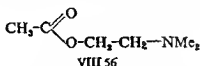
eel At pH7, in the region of the optimum for the hydrolysis of acetylcholine, the tertiary compound was hydrolysed at 60 per cent of the rate of acetylcholine, but though the cation was undoubtedly more active than the base, the base itself was quite definitely hydrolysed The inhibition of the enzyme by Neostigmine was unaffected by pH, but the effects of eserine declined in alkaline solutions With these inhibitors the ion was undoubtedly much more active than the base, but the considerable activity of eserine at pH10 must be entirely ascribed to the latter Wilson and Bergmann estimated that the affinity of the cations for the enzyme was about twenty times that of the free base

Nature of Groups in the Esteratic Site

The hydrolysis of acetylcholine by the enzymes of electric eel is markedly influenced by pH, the rate being maximal in the range pH 7 to 9 and declining

at pH values above or below these This suggests that the active centre is amphoteric, and that if the equilibrium is written

$$\overset{+}{E}H_2 \rightleftharpoons EH \rightleftharpoons E^-,$$
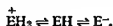
the species EH, which contains undissociated acidic and free basic groups, is the most active This idea is supported by the observation that the inhibitory effects of *TEPP* (which should not be bound at the anionic site) show a somewhat similar dependence on pH The relative independence of the effects of Neostigmine on pH must be taken to indicate that this substance is primarily bound at the anionic site rather than in the esteratic site
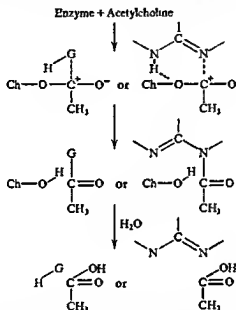
FIG VIII 8 *Hydrolysis of acetylcholine according to Wilson, Bergmann, and Nachmansohn (1950) GH represents the basic group on the esteratic site of the enzyme and may be the group shown here* $Ch = CH_2CH_2NMe_2$

From the results, Wilson and Bergmann obtained values for the dissociation constants of the reactions shown above, the pK for $\overset{+}{E}H_2$ was 7 2 and for EH was 9 3 These values were compared with the known dissociation constants of various acids in an attempt to decide what groups might be present in the enzyme They concluded that the proton accepting group in the enzyme could not be carboxylate, phosphate, guanidine, or most amino groups, but might be a special peptide –NH– group such as occurs in tyrosylarginine or phenylarginine, or the weakly basic heterocyclic nitrogen in imidazole rings The proton donating group could not be a carboxylic or phosphoric acid group, guanidinium or most phenolic groups, but might be the phenolic group in tyrosine or a simple ammonium group According to Bergmann, Wilson, and Nachmansohn (1950) the hydrolysis of acetylcholine involves

interaction between the positively polarized carbonyl atom of the ester link and the basic group in the esteratic site, and possibly a hydrogen-bond binding the ether oxygen atom. The subsequent reaction is shown in Fig VIII 8 This involves the transfer of the acetyl group to the enzyme and subsequent hydrolysis of the acetylated enzyme. The calculation of the dissociation constants of the groups in the active centre can be criticised because it does not take into consideration the effects of pH on the rate constant for the breakdown of the enzyme-substrate complex (k on page 21) Similar results, however, have been obtained with a variety of cholinesterases (and other esterases) and the conclusions appear likely to be essentially correct (review by Davies and Green, 1958)

Actions of Organophosphorus Compounds at the Esteratic Site

If the esteratic site in the active centre contains a basic group with these properties, the organophosphorus inhibitors of cholinesterases, which are all phosphorylating agents, might be expected similarly to transfer a phosphate group on to the enzyme, yielding a product from which the enzyme is not easily regenerated Wilson (1951) obtained results which indicated that this was so with the acetylcholinesterases of electric eel and *TEPP* the breakdown of the complex and regeneration of the enzyme could, however, be accelerated by substances such as choline or hydroxylamine Aldridge (1953), from a study of the effects of temperature on the breakdown of the complex formed by *Paraoxon* and the acetylcholinesterases of rabbit red cells, concluded that the activation energy for this process was about 14 4 Kcals, which is consistent with the view that the enzyme is covalently linked with the organophosphorus compound

Some of the inhibitors of cholinesterases which are carbamic esters appear to be in a position intermediate between substrates, such as acetylcholine, which form an unstable acetylated enzyme, and the organophosphorus inhibitors The observations of Wilson (1955) that the rate of dissociation of the complex formed by Neostigmine was much less than that of *Edrophonium* (page 263), and the difficulty of reversing the effects of *3113 CT* and *3152 CT* observed by Levin and Jandorf (1955 page 265), could be explained by supposing that carbamylation of the enzyme is taking place Wilson (1962) has reviewed some of the evidence that this process does, in fact, occur He estimates that at 25° the half life of the carbamyl derivative of the acetylcholinesterase of electric eel is 2 minutes, whereas for the methylcarbamyl derivative it is 39 minutes, and for the dimethylcarbamyl derivative 26 minutes

Further evidence that the organophosphorus compounds phosphorylate the enzyme was obtained by Wilson (1952), who observed that the rate of reactivation, by choline or hydroxylamine, of electric eel acetylcholinesterase poisoned by *TEPP* was the same as that when the enzyme was poisoned by diethylphosphorofluoridate In similar experiments with the acetylcholinesterases of rabbit red cells, Aldridge and Davison (1953) observed that the spontaneous return of activity at 37° was the same whether inhibition had

been caused by dimethyl(*p*-nitrophenyl)phosphate (VIII 57), dimethylphosphorfluoridate (VIII 48), tetramethylpyrophosphate (VIII 58), or O O-dimethyl S (*p*-nitrophenyl)phosphorothiolate (VIII 59) The dimethylphosphoryl enzyme had a half life at 37° of 85 to 90 minutes

Many other esterases are inhibited by organophosphorus compounds and form stable phosphoryl derivatives, di *iso*propylphosphoryl α-chymotrypsin, for instance, has actually been obtained crystalline (Jansen, Nuttting, Jang, and Balls, 1950) These phosphoryl enzymes are stable enough to be subjected to degradation and, with the di *iso*propylphosphoryl derivative of electric eel acetylcholinesterase, Schaffer, May, and Summerson (1954)

$$MeO\!-\!\underset{R}{\overset{MeO}{\bigg|}}P\!\rightarrow\!O$$

$$R = O_2N\!-\!\langle\bigcirc\rangle\!-\!O \ , \ VIII \ 57,$$
$$(R = F, \ VIII \ 48)$$

$$R = MeO \atop MeO \quad P = O \ , \ VIII \ 58,$$

$$R = O_2N\!-\!\langle\bigcirc\rangle\!-\!S \quad VIII \ 59$$
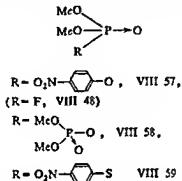
found that the only amino acid linked to phosphorus was serine Cohen, Oosterbaan, and Warringa (1955), using the cholinesterases of ox red cells inhibited by *DFP* labelled with ^{32}P, likewise found that after degradation of the complex most of the radioactivity was present as inorganic phosphate or linked to serine This is not what would be expected from the picture of the mechanism of action of the enzyme put forward by Bergmann, Wilson, and Nachmansohn (1950) The conditions for the breakdown of the protein were not such that the phosphoryl residue might have migrated, and serine itself does not react with *DFP* in aqueous solution (Ashbolt and Rydon, 1957) It appears either that the phosphoryl group migrates from the imidazole ring to an adjacent serine hydroxyl group or that the serine residue is somehow activated and modified, and that it is this modified form, not an imidazole ring, which forms the group in the esteratic site

There is considerable evidence that the first view may be correct Although the activity of acetylcholinesterase freshly treated with an organophosphorus inhibitor may be restored by the addition of a 'reactivator', such as choline, hydroxylamine, and compounds discussed below, if the inhibited enzyme is left for some time the effectiveness of the reactivator is reduced and 'aged' samples may not be reactivated at all (Jandorf *et al*, 1955, Wilson *et al*, 1955, Davies and Green, 1956, Hobbiger, 1956) This can be explained by supposing that the phosphoryl group is transferred from the imidazole ring in a histidine residue to an adjacent hydroxyl group in a serine residue If the esteratic site contains both these groups in close proximity, the phosphoryl group attached to imidazole might be regarded as activated and consequently

able to phosphorylate the serine hydroxyl group The latter might also assist in the binding of acetylcholine because of the possibility of its forming a hydrogen bond with the carbonyl oxygen atom of the ester group (Fig VIII 9), this might account for the low activation energy of acetylcholinesterases (Davies and Green, 1958)
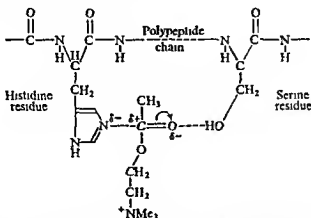
FIG VIII 9 *Hypothetical adsorption of acetylcholine at the esteratic site in acetylcholinesterase the low activation energy for the enzymic action might be due to the assistance of binding at the imidazole nitrogen atom by hydrogen bond formation between the carbonyl oxygen atom and a hydroxyl group in a serine residue (Compare with Fig VIII 8, in which hydrogen bonding involving the ether oxygen atom was considered as a possibility)*

The possibility that the serine residue is modified in the esteratic site of acetylcholinesterases (and other esterases) has been investigated by Rydon (1958) and Porter, Rydon, and Schofield (1958) A serine side-chain could cyclize, forming a Δ^2-oxazoline (Fig VIII 10), and synthetic Δ^2-oxazolines have been found to react with *DFP* in conditions in which serine itself does not react The basicity of the Δ^2-oxazoline, however, is important the 4-carbamoyl-2-phenyl compound (pK$_a$, 2 9, VIII 60) did not react, whereas the 2-phenyl compound (pK$_a$, 4 4) did, and the 2-methyl compound (pK$_a$, 5 5) was more reactive still Rydon pointed out that in many esterases the serine residue is adjacent to an aspartic acid residue, and has proposed a mechanism for the reaction which involves initial transfer of the acetyl group of the substrate (or phosphoryl group of the organophosphorus compound) to the tertiary nitrogen atom of the Δ^2-oxazoline This product can exist in two tautomeric forms (Fig VIII 10), in one of these the acyl group can be transferred to the adjacent aspartic acid carboxyl group, forming an anhydride which is subsequently hydrolysed, in the other there is no such possibility It is suggested that with the phosphoryl enzymes the tautomeric equilibrium contains predominantly the second structure, whereas with the acetyl enzyme the equilibrium lies on the other side, favouring the first structure and hydrolysis The phenomenon of 'ageing' observed with the phosphoryl enzyme is difficult to explain by this mechanism, but the idea that this

VIII 60
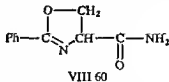
process is due to migration of the phosphoryl group from an imidazole ring to the hydroxyl group in serine has been challenged by Cohen, Oosterhaan, Jansz, and Berends (1959), who have obtained evidence that with di-iso-propylphosphorylbutyrylcholinesterases, the failure to be reactivated is associated with the gradual loss of an *iso*propyl group and formation of the mono-*iso*-propylphosphoryl enzyme.
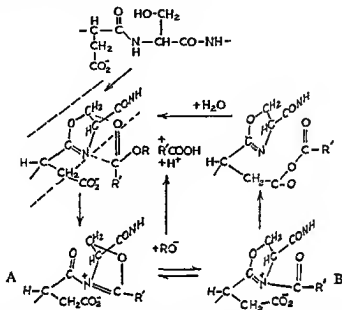
FIG VIII 10   *Hypothetical cyclization of –aspartyl serine– fragment of a polypeptide to give a Δ²-oxazoline and reaction of this with an ester If the tautomeric form A of the acyl enzyme is more stable than form B the compound should be an inhibitor (if R' is phosphoryl, for example) if form B is present to any extent hydrolysis should occur, as shown, with regeneration of the enzyme (After Rydon, 1958)*

The problem can by no means be regarded as settled either way, in spite of the attractiveness of the first theory involving the tertiary nitrogen atom in the imidazole ring of a histidine residue as the initial point of binding Oosterhaan and Van Adrichem (1958), using the relatively stable acetyl compound formed by the related enzyme, chymotrypsin, have obtained evidence which suggests that the acetyl group is bound to serine

Reactivators

Although the complex formed by organophosphorus compounds with cholinesterases is extremely stable, some degree of breakdown can be achieved by hydroxylic nucleophilic reagents, such as choline or hydroxylamine It might be expected that greater effects would be obtained if the hydroxylic reagent itself had affinity for the active centre and, in fact, better reactivators can be obtained by incorporating a quaternary group in the molecule, which

may interact with the anionic sites in acetylcholinesterases, in addition to the highly reactive hydroxyl group Wilson and Meislich (1953), demonstrated the superiority as reactivators of phosphorylated acetylcholinesterase, of
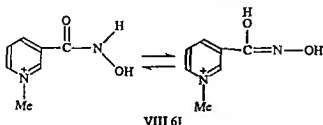
VIII 61

hydroxamic derivatives, such as nicotinhydroxamic acid methiodide (VIII 61), compared with choline or hydroxylamine themselves The more strongly basic *iso*nitrosocompounds, such as mono- and di-*iso*nitrosoacetone (VIII 62
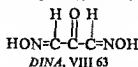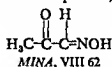
and 63), are considerably more active as are also the oximes, pyridine-2- and 4-aldoxime methiodides (VIII 64 and 65, Childs, Davies, Green, and Rutland, 1955) Higher activity still has been found in polymethylene *bis*-(4-hydroxyiminomethylpyridinium) compounds (VIII 66, Hobbiger, O'Sullivan, and Sadler, 1958, Poziomek, Hackley, and Steinberg, 1958, Hobbiger and Sadler, 1959), but Berry, Davies, and Green (1959) have shown that the second oxime group is not essential for high activity The most active compounds, trimethylene *bis* (4 hydroxyiminomethylpyridinium) (*TMB 4*, VIII 67)

2-compound, *P2AM*, VIII 64,
4-compound *P4AM*, VIII 65

and hexamethylene *bis*-(2-hydroxyiminomethylpyridinium) (VIII 68), in concentrations of 2×10^{-4}M, produced 25 per cent reactivation of diethyl-phosphoryl acetylcholinesterase of human red cells in 5 minutes An analogue

VIII 66

n = 3, *TMB 4*, VIII 67
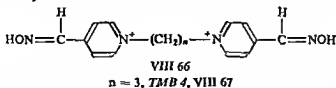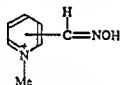
of the former containing only one oxime group (VIII 69) in the same conditions produced 25 per cent reactivation in 13 minutes, and 2-hydroxyimino-methyl-N-methylpyridinium (pyridine-2 aldoxime methiodide, *P2AM*) re-

VIII 69

VIII 68
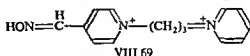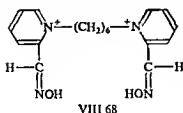
quired 60 minutes In somewhat similar experiments, Fleisher, Michel, Yates, and Harrison (1960) found that for electric eel

cholinesterase poisoned with *TEPP* the reactivation rate constant for *TMB 4* was about twenty times that of *P2AM* and for eozyme poisoned with *DFP* about ten times

That compounds of this type possess affinity for the active centres of acetyl cholioesterases is very clear because some of them are actually themselves (reversible) inhibitors of cholinesterases Also, it has been observed (Wilson, 1955, in addition see preceding paragraph) that with this type of reactivator, but not with the less active reactivators lacking a quaternary group, it is more difficult to reactivate the di isopropylphosphoryl enzyme thao the diethyl phosphoryl, this can be taken to indicate that the larger *iso*-propyl groups mask the anionic site to some extent and so impede the attachment of the reactivator

Attachment at the Active Centre

Much of what is known about the nature of the active centres in acetylcholinesterase has been obtained by direct investigation of their properties It would be expected that the picture which has been drawn should be consistent with what may be inferred indirectly by considering the chemical structure of substrates, inhibitors, and reactivators

The anioσic site appears to resemble those at which acetylcholine acts physiologically, in the neuromuscular junction, in ganglia or in postganglionic cholioergic receptors With many molecules (e g Neostigmine), increased affinity can be obtained by replacing methyl groups in the cationic head by ethyl groups, but in substrate molecules this increased affinity may be associated with a decreased rate of hydrolysis (Table VIII 6) In other molecules increased size of the cationic head does not lead to increased activity eserine and eserine methiodide, for instance, appear to be about equiactive, and with the analogues of nicotine studied by Barlow and Hamilton (1962), it was observed that quaternization often decreased anticholinesterase activity, although none of these compounds was particularly active Possibly the effect on affinity of increasing the size of the cationic head depends upon the position of the head relative to other groups in the molecule

From the stereospecificity of the hydrolysis of acetyl β methylcholine, and the lack of stereospecificity in the hydrolysis acetyl α methylcholine, it seems that the β-methyl group in the R isomer interferes with the binding of the ester group relative to the onium group (Fig VIII 11) It is also relatively easy to imagine the attachment of molecules, such as Neostigmine and *Edro phonium*, to the active ceotre, and to account for the effects of the nature of the acyl group on the hydrolysis of esters of choline The attachment of eserine itself, however, seems to be much more complicated, but further information is oeeded about the arrangement of the three fused rings in space The indo line oitrogen atom for all its apparent resemblance to the nitrogen in Neo stigmine, is less likely to be attached at the anionic site than the more basic pyrrolidine oitrogen atom It is difficult to assess how this is placed in relation to the carbamic ester group because of the fold in the molecule along the junction of the two five membered rings

Although many long chain *bis* onium salts (e g polymethylene *bis*quino-linium salts, polymethylene *bis* carbamic esters of choline, and polymethylene *bis* (4-hydroxyiminomethylpyridinium) salts) possess high affinity for acetyl-cholinesterases as inhibitors or reactivators, it is unlikely that these are combining with two active centres at once As has been pointed out already (page 133), active centres or receptors appear to constitute only a small proportion of the enzyme or receptor surface Cohen and Warringa (1953) obtained an estimate of this proportion by treating ox red cells with butyrylcholine, for which these enzymes have considerable affinity, followed by *DFP* The butyrylcholine was washed off, exposing the active centre, and the enzyme was
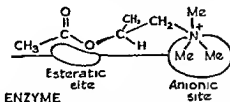
FIG VIII 11 *Hydrolysis of acetyl β methylcholine The S form is shown bound to the anionic and esteratic sites, with the R form the methyl group would interfere with binding at the anionic site, when the ester group was held at the esteratic site, and vice versa*

then treated with *DFP* containing ^{32}P A count of the radioactivity then indicated that there were 520 binding sites per cell If the area of the ox red cell is approximately 120 μ^2, this indicates 1 active centre per 2 × 10⁷ Å² It seems likely, therefore, that long polymethylene *bis* onium salts owe their high affinity to Van der Waals' adsorption along the chain in addition to binding at the anionic and, possibly, esteratic sites The observations of Berry, Davies, and Green (1959) that the second oxime group in *TMB 4* is not really necessary for high activity supports this view There may, however, in some instances, be an 'anchoring site' which is anionic in character some distance away from the active centre proper

The active centres in butyrylcholinesterases and the attachment of molecules to them have not been studied to the same extent as have those in acetyl-cholinesterases Pharmacologically the butyrylcholinesterases are relatively unimportant, although they are of clinical importance in limiting the actions of Suxamethonium (review by Harris and Whittaker, 1962) Somewhat similar enzymes appear to be important in hydrolysing atropine in some rabbits, but not in all (review by Ambache, 1955) These enzymes are apparently closely related to trypsin and chymotrypsin, the anionic site appears to be less important than with acetylcholinesterase, but the esteratic site has much in common with that of acetylcholinesterase (review by Davies and Green, 1958)

K

Relationships Between Ability to Inhibit Acetylcholinesterases and Pharmacological Properties

At the beginning of this chapter evidence was presented that some of the properties of eserine can be ascribed to its ability to inhibit acetylcholinesterases It is necessary to see whether this is true for other physiological properties, such as the reversal of neuromuscular block produced by (+)-tubocurarine and the improvement of conduction at the neuromuscular junctions of patients suffering from *myasthenia gravis*, also whether it is true for other inhibitors of acetylcholinesterase

Although it is very convenient to classify all the inhibitors of acetylcholinesterases discussed in this chapter as 'Anticholinesterases', this label may be extremely misleading because it takes no account of any other properties the compounds may have Substances such as eserine and Neostigmine exhibit a high degree of specificity and affect cholinesterases in concentrations very much less than those which produce any direct action on, for instance, the neuromuscular junction The work of Blaschko, Bulbring, and Chou (1949), Smith, Cohen, Pelikan, and Unna (1952), Pelikan, Smith, and Unna (1952), Nastuk and Alexander (1952), Hobbiger (1952), Wilson (1955), Katz and Thesleff (1957) and Nastuk and Alving (1958) all supports the view that the ability of eserine, Neostigmine, and Edrophonium to reverse neuromuscular block produced by (+)-tubocurarine is directly a consequence of their inhibition of the destruction of acetylcholine Riker *et al* (1957, 1959) has argued that because these substances give rise to antidromic action potentials in the pre synaptic fibres they may act at the nerve terminal The ability of Neostigmine to give rise to antidromic action potentials was observed by Masland and Wigton (1940) but it is possible that these arise not from an action by the Neostigmine at the nerve terminals but by the action of acetylcholine Even if these substances themselves have a pre synaptic action on the nerve-endings, it seems very doubtful whether, with these compounds, this is relevant to their ability to reverse the effects of (+) tubocurarine

In *myasthenia gravis* the relationship between the ability of compounds to alleviate the muscular weakness and their ability to block the destruction of acetylcholine may depend upon the cause of this muscular weakness The condition is consistent with the failure in transmission at certain neuromuscular junctions due to too little acetylcholine This could be because there is too much acetylcholinesterase, too little acetylcholine released, or to decreased sensitivity of the receptors Removal of the thymus gland is beneficial in certain patients, but the idea that this gland is producing a circulating neuromuscular agent is difficult to accept because of the way in which the disease is often restricted to individual muscles Myasthenic patients are particularly sensitive to (+) tubocurarine and resistant to Decamethonium (Churchill Davidson and Richardson, 1952), and the possibility that the disease is caused by a change in the sensitivity of the receptors in the end-plate has been considered by Zaimis (1952) An electrophysiological comparison of muscle obtained from myasthenic patients with normal muscle by

Dahlback, Elmqvist, Johns, Radner, and Thesleff (1961) reveals a significantly lower incidence of miniature end plate potentials in the former. The results strongly indicate a deficiency in the release of acetylcholine from the nerve-endings. The effectiveness of a drug in alleviating the muscle weakness could, therefore, to be related directly to its ability to preserve acetylcholine from destruction at the neuromuscular junction. If the acetylcholinesterase at the junction is blocked, such acetylcholine as is released should have more chance of causing an end plate potential. If the fact that compounds such as *Ambenonium* and *Methoxyambenonium* are concentrated at the neuromuscular junction is taken into consideration, there does not appear to be any evidence that these compounds are not acting at the myasthenic neuromuscular junction only because of their effects on acetylcholinesterase.

The organophosphorus compounds, such as *DFP*, affect many sites besides the active centres of acetylcholinesterase. This can be seen, for instance, in the need in the experiments of Cohen and Warringa (1953) to treat the ox red cells with unlabelled *DFP* (the active centre being protected by butyrylcholine) before applying labelled *DFP*. There seems little doubt that most of the immediate effects of these compounds are due to block of acetylcholinesterase, but certain effects, particularly the delayed effects on nerve fibres, are not related to this action (review by Holmstedt, 1959). Tri-o-cresyl phosphate (VIII 70), for instance, which is a not particularly potent inhibitor of butyrylcholinesterase and even less active on acetylcholinesterases (Earl and Thomson, 1952), causes, in time, a drastic demyelination of nerve-fibres in the spinal cord, leading to permanent paralysis. Davies, Holland, and Rumens (1960) tested the ability of many compounds to produce this neurotoxicity and showed clearly that it was not related to their effects on cholinesterases.

The effects of reactivators in overcoming the immediate effects of organophosphorus inhibitors of acetylcholinesterases seem likely to be directly related to their ability to remove phosphoryl groups from the enzyme (see, for example, Rutland, 1958), but the situation may be complicated by the poor penetration of some of the compounds which are quaternary ammonium salts into the central nervous system (Holbiger and Sadler, 1959)

Conclusion

It can be shown that many compounds produce pharmacological effects indirectly by acting on acetylcholinesterases. It is perfectly justifiable to attempt to interpret their actions at the active centres in the enzymes at a molecular level. It is reasonable to suppose that the behaviour of the receptors in the enzyme may not be entirely different from that of the receptors in the neuromuscular junction, in ganglia, etc., and the results with the enzyme accordingly justify attempting to discuss events at these other receptors also at a molecular level in a similar way.
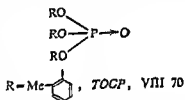
IX

Actions at Adrenergic Receptors

Physiological Events

As was pointed out in Chapter IV, the actions of the sympathetic transmitter can be divided into excitatory and inhibitory

At sites such as the receptors in the nictitating membrane of the cat, the transmitter causes a contracture, and it would be expected that this is associated with depolarization of the muscle membrane

At sites where the action of the transmitter is inhibitory, it is not often clear whether the transmitter is affecting cells which are different from those affected by parasympathetic stimulant drugs and which act mechanically in the opposite sense, or whether the transmitter is acting on the same cells and directly modifying the actions of the parasympathetic stimulants The actions of adrenaline and acetylcholine on heart muscle, for example, can reasonably be accounted for by supposing that both substances are acting on the same cells, but that, whereas acetylcholine increases the permeability to K^+ ions, adrenaline affects the permeability to Na^+ and other ions (review by Hutter, 1957) Acetylcholine accordingly increases the resting membrane potential and tends to depress activity, whereas adrenaline tends to depolarize the membrane and to increase the excitability The state of the muscle at any particular time thus depends upon the algebraic sum of the effects on ionic permeability produced by the sympathetic and parasympathetic transmitters

In smooth muscle it is possible that there is a similar state of affairs, but

with the actions of acetylcholine and adrenaline reversed, acetylcholine producing depolarization (Chapter VII) and adrenaline affecting the resting potential of the membrane Some electrical evidence for this has been obtained in the experiments with the *taenia coli* of the guinea pig already described on page 186 With this tissue it seems likely that adrenaline and acetylcholine both act on the same cell The action of adrenaline, however, appears to be dependent on its ability to modify the effects of acetylcholine, the relaxation is not produced actively, but by interference with the normal tone of the preparation which is maintained by parasympathetic stimulation With blood vessels the situation is more complicated Although there is no parasympathetic nerve-supply, there may be cholinergic sympathetic connexions, and it is by no means clear whether the various effects of adrenaline can be ascribed to actions on these cholinergic vessels (as has been suggested for the *taenia coli*) or whether it acts on a separate set of vessels altogether

The physiological event at adrenergic receptors in smooth muscle accordingly appears to be associated with, and probably the consequence of, electrical events at the muscle membrane In this respect the situation resembles that at cholinergic receptors Convincing evidence for the quantal release of sympathetic transmitter at nerve-endings has not yet been forthcoming, but Burnstock and Holman (1960, 1962) have obtained results which could be interpreted in this way in experiments with micro-electrodes inserted into single fibres of the *vas deferens* of the guinea pig (which contracts in response to sympathetic stimulation)

The storage of the sympathetic transmitter has been shown at many sites to occur in 'chromaffin' cells, so called because they become coloured on exposure to oxidizing agents The transmitter is found associated with adenosine phosphates (the cations and anions being in apparently equivalent amounts) in discrete granules which can be separated by centrifugation (Blaschko, Hagen and Hagen, 1957), and which are recognizable in electron micrographs, being about 100 μ in diameter (Hagen and Barrnett, 1960) The alkaloid reserpine (page 327) interferes with the storage mechanism, causing release of stored material and subsequent depletion of the stores Other substances, such as tyramine (page 306) also affect the storage process There are, therefore, a number of steps intermediate between the arrival of an impulse at the sympathetic nerve-endings and the release of the sympathetic transmitter Burn (1961) has suggested that these nerves are actually cholinergic and that, as in the adrenal medulla (page 81), the first step is the release of acetylcholine, this then acts on processes which result in the release of sympathetic transmitter from stores Although this hypothesis conveniently explains many experimental results, it cannot be regarded as being an established fact

In addition to its effects on smooth muscle, adrenaline produces biochemical changes, giving rise to increased glycolysis These metabolic effects appear to be due, at least in part, to alteration of the enzymic conversion of adenosine triphosphate to cyclic 3 5 adenosine monophosphate and leading eventually to increased conversion of glycogen to glucose 1-phosphate (review by

Sutherland and Rall, 1960) These processes occur predominantly in the liver, but can also occur in muscle, and it has even been suggested (Bülbring, 1960) that the relaxation of smooth muscle is a consequence of these metabolic changes occurring in the muscle membrane and stabilizing it, making it less excitable

Identity of the Sympathetic Transmitter

Although the term 'adrenergic' has been used to describe the receptors which interact with the sympathetic transmitter, this latter is not a single substance, adrenaline, but a mixture At least two substances are present (page 81), adrenaline and noradrenaline, and the proportions and amounts of these vary from site to site and also depend upon the intensity of nervous activity, being different if the nerve is stimulated only occasionally from what it is if the stimulation is persistent There is the possibility that even more substances may be present in the mixture in certain circumstances Unlike acetylcholine, which is formed from, and broken down to, substances which are virtually inactive (choline and acetic acid), adrenaline is formed from, and broken down to, complex products, some of which might well be effective themselves Blaschko (1939) and Holtz (1939) suggested that adrenaline might be formed from tyrosine (Fig IX I), and this scheme now appears to be correct (reviews by Schümann, 1960, Kirshner, 1960, Vane, 1962) The substance 3 4-dihydroxyphenylethylamine ('dopamine', IX I), the precursor
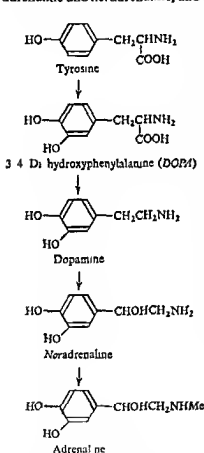
FIG IX I *Formation of adrenaline and noradrenaline from tyrosine*

Dopamine, IX.1
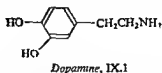
of noradrenaline, is stored in the granules, possesses appreciable activity (page 300) and accordingly may well be a constituent of the transmitter mixture It is interesting that, although adrenaline is formed by methylation of *nor* adrenaline, histological evidence indicates that in the adrenal medulla the two substances are stored in granules in two different types of cell In other sites, however, where the transmitter is predominantly *noradrenaline*, there is *no* evidence for the separate storage of the two substances

The breakdown of sympathetic transmitter is nothing like as rapid as the hydrolysis of acetylcholine but, conversely, if the transmitter has access to the blood-stream, the effects do not persist beyond seconds or, at the most, minutes Axelrod (review 1960), using isotopically labelled adrenaline and noradrenaline, has shown that after an intravenous injection much of the material is rapidly and selectively taken up by specific sites, such as the heart and the spleen Roughly half is metabolized in 5 minutes, but after this period the substances disappear more slowly, appreciable amounts being present several hours later In isolated tissues, or at sites with a poor blood supply, the effects are much more persistent Interference with the destruction of the transmitter could greatly prolong its action, and there are many compounds which could produce sympathomimetic effects by acting in this way In principle, the situation is comparable with the actions of substances like eserine in preventing the destruction of acetylcholine, but there is a big difference in that the breakdown of adrenaline and noradrenaline proceeds in a number of ways and there has been much confusion about the precise route or routes followed
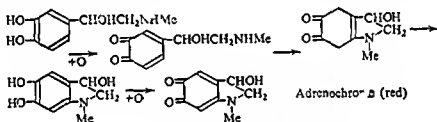
FIG IX 2   *Formation of adrenochrome*

The phenolic groups could be oxidized to an *o* quinone and hence to adrenochrome (Fig IX 2), this can readily be observed if a dilute neutral solution of adrenaline or noradrenaline is left exposed to air for an hour or so The amino group could be oxidized to the imine and hence, through the aldehyde, to 3 4-dihydroxymandelic acid (Fig IX 3) The phenolic groups could be
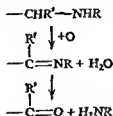
FIG IX 3   *Oxidation of amino groups although there is no chemical reason why the reaction should not proceed when R is methyl or ethyl, it does not do so and these α substituted compounds inhibit the actions of these enzymes (see page 339) When R is hydrogen the aldehyde formed may be oxidized to the carboxylic acid, but this can be stopped by addition of cyanide ions*

methylated and/or conjugated with substances such as glucuronic acid or sulphuric acid Another possibility which has seriously to be considered is

that transmitter may be taken up at other sites in the body (e g returned to stores) without being destroyed

In the past, trouble has been experienced partly because of the inadequacy of analytical methods used for identifying the various metabolites and, in consequence, the need to give abnormally large doses, partly because the complexity of the breakdown process was not appreciated (the importance of O methylation, for instance, has been overlooked until recently), and partly because inferences have often been made indirectly from the changes in metabolism produced by substances which were supposed to block specifically only one metabolic pathway (e g amine oxidase) A further complication has been that the enzymes involved have not been purified and characterized satisfactorily and it is, accordingly, difficult to transfer information obtained *in vitro* to the situation *in vivo*

From the evidence now available from experiments in man with small amounts (e g 50–300 µg per subject) of isotopically labelled adrenaline and noradrenaline (reviews by Axelrod, 1960, Kirshner, 1960), it seems clear that the initial uptake of adrenaline and rapid disappearance is associated with extensive O methylation (Fig IX 4) and that this is the most important meta-
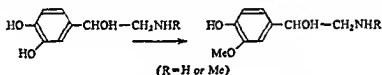
FIG IX 4   *Inactivation of noradrenaline and adrenaline by catechol O methyl transferase*

bolic pathway The O methyl compound (*Metanephrine*, IX 2) may subsequently be deaminated by amine oxidase and react further This is not the only

Metanephrine, IX.2
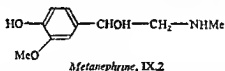
route, however, for although 68 per cent of the adrenaline was O methylated, 23 per cent was deaminated and oxidized or reduced before O methylation The residual 9 per cent was excreted either unchanged or conjugated *Nor* adrenaline appeared to be metabolized in a similar fashion The process of O methylation drastically reduces the activity of adrenaline (page 313) and the other products of the breakdown are inactive

The effects produced by sympathetic stimulation can accordingly be ascribed to the actions of mixtures containing adrenaline, noradrenaline, and possibly some 3 4-dihydroxyphenylethylamine The composition varies, but at most nerve-endings noradrenaline appears to be the major component This substance alone, however, cannot be regarded as the sympathetic trans mitter for the receptors in the tissues also vary in sensitivity, and at some of them (the β-receptors on page 291) noradrenaline is only feebly active Con-

tranrwise, adrenaline alone cannot he regarded as the sympathetic transmitter, although for a long time it was thought to be so, because at other sites (the α receptors) its effects are different from those of the transmitter, which contains a high proportion of noradrenaline At postganglionic sympathetic nerve-endings there is neither a single transmitter nor a single type of receptor, and in discussing the actions of drugs it is important to indicate what is known about the type (or types) of receptor in the test preparation

Types of Receptors

The classification of adrenergic receptors into α and β by Ahlquist (1948) was based on the sensitivity of different tissues to different drugs (Table IX 1) In a

TABLE IX 1

Classification of Adrenergic Receptors Activities of Catecholamines
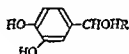
Tissue	Effect	Compounds arranged in order of potency, R =
Blood vessels* Uterus† Nictitating membrane Ureter Intestine	Constriction Stimulation Contraction Contraction Relaxation	(−)-CH₂NHMe (adrenaline) > (±)-CH₂NHMe > (±)-CH₂NH₂ (noradrenaline) > (±)-CHMeNH₂ > (±)-CHMeNHMe > (±)-CH₂NHisoPr (Isoprenaline)
Blood vessels* Uterus† Heart	Dilatation Relaxation Increase in rate and force of beat	(±)-CH₂NHisoPr (Isoprenaline) > (−)-CH₂NHMe (adrenaline) > (±)-CHMeNHMe > (±)-CH₂NHMe > (±)-CHMeNH₂ > (±)-CH₂NH₂ (noradrenaline)

* Different sets of vessels
† The response of the uterus depends upon its condition, the normal uterus may give a biphasic response stimulation followed by relaxation

Ahlquist (1948)

variety of tissues (−) adrenaline was the most active of the compounds tested and (±) Isoprenaline (IX 3) the least active the receptors in these were
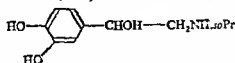
Isoprenaline IX 3

thought to be similar and called the α type In other tissues (±) Isoprenaline was the most active and (±) noradrenaline the least active the receptors in these were also thought to he similar and termed the β type On most of the

tissues of the first group, the effect produced was excitation (contracture), and on most of those in the second group the effect was inhibition (relaxation), but the receptors cannot be classified simply according to the nature of the effect produced because relaxation of the intestine appears to be produced by an action at α-receptors, and increase in the rate and force of the beating of the heart by an action at β-receptors

The classification of the receptors in this way into two types is supported by the properties of antagonists (review, Furchgott, 1960) Most of these are effective only on tissues containing α receptors, but there have recently been developed substances which block mostly only β-receptors The receptors sensitive to the action of adrenaline in stimulating glycogenolysis and those involved in relaxation of the intestine do not appear to fit into the general classification, and have been termed γ- and δ receptors respectively by Furchgott (1959) Both the phosphorylase activity of heart muscle and the effects of adrenaline, however, are blocked by the same antagonist (Mayer and Moran, 1960), and it is therefore possible that the γ-receptors are identical with the β-receptors in the heart Evidence obtained by Furchgott (1960) indicates that the receptors in the gut may be a mixture of both types, α- and β-, and not necessarily the separate δ type

There is, therefore, from the experiments with antagonists, only justification for postulating two sets of receptors, α- and β-, but the inclusion of the receptors in the heart in the β-type is difficult to accept on physiological grounds Relaxation appears to involve stabilization of the membrane (whether a consequence of increased phosphorylase activity or not), whereas in heart muscle substances like adrenaline tend to depolarize and increase the excitability In these circumstances it does not seem justifiable to suppose that the receptors have the same structure, even though both are blocked, like the hypothetical γ-receptors, by the same drug It would seem more logical to classify the β-type receptors in heart muscle separately from other β receptors, at least until such a precaution is shown to be superfluous

Preparations Containing α-Receptors

Isolated Preparations

Isolated preparations of tissues which contract in response to sympathetic stimulation have been developed only relatively recently The action of adrenaline on the *vas deferens* was noted by Waddell (1916), but it was not until the work of Leach (1956) that the possible use of this preparation for testing adrenergic drugs and antagonists came to be appreciated Leach used the *vas deferens* of the guinea-pig mounted in an isolated organ bath A modification in which the nerve supply is dissected out and can be stimulated, due to Hukovic, is described by Boyd, Chang, and Rand (1960) This is suitable for experiments with intracellular micro electrodes with which electrical events on single fibres can be investigated (Burnstock and Holman, 1962) The simple preparation without the nerve supply has been used extensively for testing agonists and antagonists by Koopman (1960, results quoted by Ariëns, 1960) The muscles contract in response to acetylcholine and histamine as well as to

adrenaline and noradrenaline, but the site of action of the test drug can be determined by investigating the **effects of antagonists** (Leach, 1956, used atropine, Mepyramine and **Piperoxan**, an antagonist at the α type adrenergic receptors) Noradrenaline was active in concentrations of around 10^{-6}M, adrenaline was slightly less active, and acetylcholine and histamine less active still

The seminal vesicles of the guinea pig can similarly be employed, without the nerve supply, as a simple isolated preparation (Stone and Loew, 1952) As with the vas deferens contractures are also obtained in response to acetylcholine and histamine as well as to adrenaline and noradrenaline (all in concentrations of around 10^{-5} M)

Lewis and Koessler (1927) described a method of mounting strips of artery (obtained from a large animal, such as a cow) in an organ bath, and Furchgott and Bhadrakom (1953) have modified this for use with smaller strips, such as those of rabbit aorta This preparation is extremely sensitive to adrenaline and noradrenaline, detectable contractures being produced by concentrations as low as 10^{-9} M Contractures are also produced by histamine and acetylcholine, but much higher concentrations are needed, about 10^{-7} M histamine and 10^{-6} M acetylcholine

Rosenblueth and Cannon (1932) described the effect of adrenaline in causing contracture of the nictitating membrane of the anaesthetized cat, prepared as described by Querido (1924) Recently, Thompson (1958) has described how this tissue may be mounted as an isolated preparation The membrane contains two sets of muscles, medial and inferior, but the responses to stimulant and antagonistic drugs are qualitatively indistinguishable, although bigger responses are always obtained with the medial muscle Detectable re sponses were obtained with adrenaline and acetylcholine in concentrations of around 10^{-7} M, with noradrenaline in concentrations around 10^{-8} M and with higher concentrations of other substances, such as nicotine

Bülbring and Hooton (1954) have described an isolated preparation of the *sphincter pupillae* muscles, which contract in response to sympathetic stimulation, dilating the pupil of the eye This preparation has been used to record electrical events with intracellular micro electrodes, but has not been used much for the study of the effects of drugs

The vasoconstrictor actions of substances like noradrenaline and adrenaline can be demonstrated in isolated perfused vessels, such as the perfused rabbit ear (Pissemski, 1914, Schlossmann 1927, Burn, 1952) and the perfused rat hindquarters (Fastier and Smirk, 1943, Burn, 1952) In these the animal is anaesthetized, the arterial supply of the vessels is cannulated, and the ear or hindquarters separated from the rest of the animal (which is then killed) The effects of drugs can then be studied by observing the changes in the rate of flow of the perfusion fluid In these isolated preparations there are no connexions with the central nervous system, and consequently the results may be more informative than similar experiments on the effects of drugs on the rate of flow of perfusion fluids or blood through the vascular beds of intact animals Even in the rabbit ear, however, there are many types of vessel which

have different sensitivities to a compound such as adrenaline Although adrenaline produces vasoconstriction of the vessels of the rabbit's ear as does *noradrenaline* in higher doses, the tissues cannot be regarded as containing only α-receptors Gowdey (1948), for example, found that when the preparation had been treated with *Tolazoline* (which blocks α-receptors, see page 329), adrenaline produced vasodilatation One practical complication with this type of preparation is that if an overdose of a vasoconstrictor drug is given, the preparation is useless, because the perfusion stops altogether and the drug cannot be washed out of the tissues

Intact Preparations

The effects of adrenergic drugs on blood-vessels are extremely complex and difficult to interpret (see, for example, Ginsburg and Cobbold, 1960, Folkow, 1960), and the overall effects on the blood-pressure are even more complex since the actions on the heart as well as on the peripheral resistance are involved Much of the available information about the activity of compounds at α-receptors, however, is based on experiments on the blood-pressure of spinal or anaesthetized animals and even in man One complication in these is the possibility of *reflex vagal stimulation* in response to a rise in pressure, with the result that the rise is followed by a fall accompanied by slowing of the heart This can be prevented in experiments in animals by administering atropine or, better, by cutting the vagus nerve The effect on the blood-pressure, however, may be the consequence of actions on both α- and β-receptors, some of which act in opposite ways Adrenaline, for instance, by its action on the heart and the α-receptors in the arteries and some of the peripheral blood-vessels should raise the pressure, but by dilating other vessels, particularly those in voluntary muscle, it should tend to lower the pressure By administering a drug which blocks the α receptors (Dale, 1906, used ergot alkaloids, see page 320) the fall in pressure produced by the actions on the β-receptors is unmasked In these circumstances, the preparation could be used for assessing activity at β-receptors, though this is complicated by the effects on the heart, which tend to raise the pressure If a substance were administered which blocked only the β receptors and the heart, the preparation could then be used for assessing activity only at α receptors Such substances are now known (page 315), but have not been used for this purpose to any extent

The *in vivo* estimation of activity at α-receptors should be possible by comparing doses of compounds producing comparable degrees of dilatation of the pupil, because this is caused by contraction of the sympathetically innervated *sphincter pupillae* Some use has been made of the sympathetically denervated cat's eye, in which the sensitivity of the preparation has been greatly increased by surgical removal of the superior cervical ganglion some time before the experiment (Cannon and Rosenblueth, 1935, Burn and Hutcheon, 1949) The preparation is too elaborate, however, and the range of doses which can be given is too narrow for extensive quantitative testing

The estimation of activity at α-receptors in man is only possible if the com-

pound bas no activity at β-receptors The blood-pressure can easily be recorded, and so can the passage nf blood through the blood-vessels of the limbs, because this produces changes in volume which can be measured by a plethysmograph Changes in either blood-pressure nr limb-volume, however, can be brought about by actions at either type nf receptor and it is not feasible in man to attempt to block β-receptors alone

Preparations Containing β-Receptors:

Isolated Preparations·

Of the isolated preparations suitable fnr testing activity at β-receptors, the isolated uterus has been longest in use Kehrer (1906) described an isolated preparation of cat uterus, and many nf the compounds studied by Barger and Dale (1910) were tested for their nbility to relax this preparatinn De Jalon, Bayo, and De Jalon (1945) used the isolated non-pregnant uterus of the rat, made to contract regularly by n dose of acetylcholine given at fixed intervals The effects of doses nf adrenaline in reducing the size of these contractions may be compared with those of n test solutinn and an accurate assay performed Gaddum and Lembeck (1949) made use of this preparation to determine the amount nf adrenaline in mixtures of adrenaline with *noradrenaline* because the latter bas very little activity on this preparation

The action of drugs in relaxing brnnchial muscle may conveniently be tested nn the isolated guinea-pig tracheal chain described by Castillo and De Beer (1949) Although the actual brnnchial muscles are very short, a number (usually a dozen) of ring-like sections of trachea can be joined together with thread to make a chain This is then mounted in an organ bath, and contractions can be produced with drugs such as acetylcholine and histamine, and the ability to cause relaxation measured in the same way as with the rat uterus

If desired, effects can be observed nn the whole broncbial tree, including the lungs In the preparation described by Sollmann and Von Oettingen (1928) perfusion fluid was passed down the trachea, but in subsequent modifications (for example, by Fastier and Reid, 1952, and Arunlaksbana and Schild, 1959) these passages are perfused with air and the lungs tissue kept alive by the perfusion of physiological saline through the pulmonary artery

Intact Preparations

The *in vivo* assessment of activity at β-receptors by studying effects on blood-pressure in the presence of a substance which blocks actions at α-receptors has already been mentioned Effects on the passage of air through the lungs in anaesthetized animals can also be observed, and Dornborst and Herxheimer (1958) bave been able to make observations of these effects in conscious man It is possible to measure the pressure in the oesophagus (Dornhorst and Leathart, 1952), and this together with a record of the chest movements can he used to compute the force needed to expand the lungs Results obtained by this technique should largely indicate activity on the β-receptors of bronchial

muscle, provided there are no major changes in general blood pressure, effects on the central nervous system, or other obvious complications.

Intestine

The *in vitro* activity of compounds on the receptors (supposed to be of both types) in the intestine may be assessed by using the isolated preparations already described (page 144). As with the rat uterus, it is necessary to produce regular contractions by the addition of a standard dose of acetylcholine (or some other agonist), the effects of the compounds in reducing these are used to compare their activity. In the modification of this preparation described by Finkleman (1930), however, rabbit intestine is used, this has high spontaneous activity and regular contractions may be obtained without the addition of any agonist. Either stimulation of the sympathetic nerves (which are easily accessible and can be placed across electrodes in the organ bath), or the addition of adrenaline, causes the spontaneous contractions to stop and the whole muscle to relax. This preparation is particularly useful for testing antagonists and for distinguishing between those which antagonize the actions of adrenaline and those which prevent the *release of sympathetic transmitter from the nerve-endings* (page 340).

Vane (1957, 1960) has described an isolated preparation of the muscles of the fundus of the rat's stomach, which contracts in response to some substances (acetylcholine, histamine, and 5 hydroxytryptamine) and relaxes in response to adrenaline, *noradrenaline*, and isoprenaline. It is possible that, unlike the intestine, this part of the digestive tract contains only the β type of adrenergic receptor.

Heart

Some preparations of heart muscle have already been described (page 191), from which information about electrical events may be obtained with intracellular microelectrodes. These have not, in fact, been much used for the quantitative comparison of the activity of adrenergic drugs. The spontaneously beating auricles of the guinea pig or rabbit have been used to some extent, as have perfused whole hearts, such as the preparations of Straub and Langendorff. With the latter, information may also be obtained about the effects on the coronary vessels, as well as on the rate and force of the heart beat. The effects may easily be observed in man by following the pulse-rate, but the quantitative comparison of the relative activities of adrenergic drugs in this way would be complicated and hazardous.

Methods for Assessing Activity

Agonist activity can only be expressed in terms of that of a standard drug, usually (−) adrenaline, because this affects both α- and β receptors. Sometimes, however, it is necessary to use isoprenaline as a standard for activity on β receptors. The activity of antagonists could be expressed in terms of the equilibrium association constant and this procedure has been followed by Koopman (1960) and Ariëns (1960). Most of the more active antagonists,

however, do not act by competition and activity should be expressed in terms of pA_h or pD_2' (page 16)

Methods for the Quantitative Estimation of Adrenaline and Noradrenaline

The identification of the constituents in the sympathetic transmitter has only been possible because of the development of quantitative methods for the separate estimation of the constituents These methods may be based on either biological or chemical tests but, with either type, the results should be more reliable if the amines are first separated by chromatography (James, 1948, Crawford and Outschoorn, 1951) This procedure, however, is not essential, and biological methods for the assay of adrenaline in mixtures containing *noradrenaline* have been developed (e g Gaddum and Lembach, 1949) which do not involve the separation of the two The method makes use of the much greater activity of adrenaline at the β receptors in the rat uterus, and the approximately equal activity of the compounds at the receptors in the rat colon Assay of the mixture on the rat uterus accordingly indicates the amount of adrenaline present and the amount of *noradrenaline* is estimated by subtracting this quantity from the result of the assay on the rat colon The value of the results obtained must be assessed by statistical analysis and the experiments take several hours, but only very small amounts of material are needed

Chemical methods are based upon the production of coloured or fluorescent products (Von Euler and Hamberg 1949, West, 1949, Bowman, Caulfield, and Udenfriend, 1955, Von Euler and Floding 1955) Although these are less time-consuming than the biological methods, they are not so sensitive and, unless the individual amines are separated by chromatography, it may be questioned how far the tests are specific Estimates based on absorption or emission figures at two different wavelengths or pH values could conceivably be greatly affected by the presence of impurities

Uses of Adrenergic Drugs and their Antagonists

There is a limited use for adrenergic drugs to start the heart which has stopped, for instance, during an operation In conditions of shock when the circulation is poor, the intravenous infusion of *noradrenaline* brings about an improvement by causing vasoconstriction and partly by its direct effects on the heart For this purpose substances which act principally at α receptors are more suitable than those which also affect β receptors (and would also cause some vasodilatation) The vasoconstrictor properties of adrenaline and *noradrenaline* are also widely made use of to prolong the actions of local anaesthetics by reducing their rate of removal by the blood stream from the site of injection

The most important use of adrenergic drugs is for relaxing bronchial muscle in asthmatic attacks Whatever the cause (release of histamine and/or of other substances) an asthmatic attack results in acute bronchoconstriction and this can be overcome by the action of adrenergic compounds which affect the β-receptors Unfortunately, with substances such as adrenaline and Isoprenaline, the bronchodilatation is accompanied by effects on the heart which may

be unpleasant With the latter in particular, the increased heart rate is associated with intense vasodilatation, and though there is an overall fall in blood-pressure, there is a large difference between the pressure in systole and in diastole with each heart beat

Adrenergic blocking agents might be expected to be valuable in the treatment of conditions of raised sympathetic tone, but have not been particularly useful, largely because the compounds available have unpleasant side effects Greater use has been made of ganglion blocking agents (Chapter VI), and there have recently been discovered a number of compounds which selectively block the release of sympathetic transmitter from nerve endings These are sympatholytic drugs, not adrenergic blocking agents, they do not block the actions of adrenaline or noradrenaline, but have been widely used in attempts to block the release of sympathetic transmitter in patients with high blood pressure due to a raised sympathetic tone

AGONISTS

Activity and Stereospecificity of Adrenaline and Noradrenaline

The effects of extracts of the adrenal gland in raising the blood pressure were observed by Oliver and Schaefer (1895) Abel and Crawford (1897) succeeded in isolating a benzoyl derivative of the active principle of this extract, but it was Takamine (1901) who first isolated adrenaline itself The compound was synthesized by Stolz (1904) and the resolution of the optical isomers was achieved by Flächer (1909) The (−)-isomer is much more active than the (+)- (Table IX 2) and has been shown to have the R configuration (Fig IX 5, Pratesi, La Manna Campiglio, and Ghislandi 1958)

$$\overset{\displaystyle CH_2NHR}{\underset{\displaystyle X}{\overset{\displaystyle |}{\underset{\displaystyle H}{\diagup}}\overset{\displaystyle \diagup}{\underset{\displaystyle OH}{\diagdown}}}}$$

FIG IX 5   *Absolute configuration of noradrenaline and adrenaline*

$$X = HO\!\!-\!\!\!\left\langle \rule{0pt}{12pt}\right.\!\!\!-\!\!\!\left.\rule{0pt}{12pt}\right\rangle\!\!-, \quad R = H \;\; R \; noradrenaline \;(-),$$
$$\qquad\qquad\qquad\quad R = Me, \; R \; adrenaline \;(-)$$
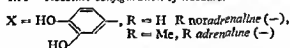
Noradrenaline was prepared by Stolz and Flächer (1904) and has been resolved by Tullar (1948) As with adrenaline, the (−)-isomer is more active than the (+)- and has the R configuration (Fig IX 5, Pratesi, La Manna, Campiglio, and Ghislandi, 1959)

From the figures in Table IX 2 it would seem that the receptors in the rat uterus are less stereospecific than elsewhere, as well as being less sensitive to noradrenaline The two properties, however, are not connected because the receptors in the guinea pig lungs also appear to be much more sensitive to adrenaline than to noradrenaline and yet are highly stereospecific The figures also show that although, in general, noradrenaline is more active than adrenaline at α receptors and less active at β-receptors, on the heart and intestine the difference is not great

TABLE IX 2

Properties of Isomers of Adrenaline and norAdrenaline

Tissue	Receptor type (?)	Equipotent molar ratios		
		Of (+)-adrenaline relative to (−)	Of (+)-nor-adrenaline relative to (−)	Of (−)-nor-adrenaline relative to (−)-adrenaline
Blood pressure rise				
Dog	α	15 (A and M) 12-15 (C)	27 (L)	0·6 (L)
Cat	α	20 (T) 18 (SS)	—	0 7-0 8 (B), 0 4 (W), 0 7 (S)
Rabbit	α	30-40 (F)	—	—
Perfused rabbit ear	α	—	12-18 (L)	1 5-2 5 (L), 1-3 (G)
Rat uterus	β	—	4 (L)	30 (L), 75-300 (G)
Perfused guinea pig lungs	β	45 (LE)	70 (LE) 60 (L)	58 (LE) 17 (L)
Dog heart (*in situ*)	—	—	27 (L)	0 6 (L)
Guinea pig intestine	—	—	27 (L)	1 5 (L)
Rabbit intestine	—	—	—	1 (W) 3 (G)
Guinea pig colon	—	—	—	2 (G)
Rat colon	—	—	—	0 2-1 (G)
Rat fundus strip	—	—	250 (V)	3 7 (V)

A and M = Abderhalden and Müller (1908)   C = Cushny (1908, 1909)   T = Tainter (1930)   F = Fromherz (1923)   L = Luduena Ananenko Siegmund and Miller (1949) B = Barger and Dale (1910)   S = Schultz (1909)   G = Gaddum Peart and Vogt (1949) LE = Luduena Von Euler, Tullar and Lands (1957)   W = West (1947)   V = Vane (1960) SS = Swanson Scott Lee, and Chen (1943)

Effects of Altering the Substituents on the Nitrogen Atom

The effects of the replacement of the methyl group in adrenaline by larger alkyl groups are shown in Table IX 3 Although the higher homologues of 3 4-dihydroxyphenylethylamine were studied by Barger and Dale (1910) and the *n* propyl compound (IX 4) was found to have very low pressor activity,
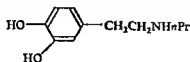
IX 4

the higher homologues of adrenaline were not studied until the work of Konzett (1941) The *iso* propyl compound, Isoprenaline, was found to be particularly active in dilating the bronchi and reducing the blood pressure, but increased the rate of beating of the heart Very many compounds of this type were prepared and tested but, with the possible exception of the *cyclopentyl*

TABLE IX 3

Properties of N-Substituted Derivatives of (±)-norAdrenaline
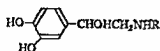
HO—⟨benzene ring⟩—CHOHCH₂NHR

	Equipotent molar ratios relative to Isoprenaline		
	Dilatation of bronchi, guinea-pigs	Fall in blood pressure, anaesthetized dogs	Rise in heart-rate, anaesthetized dogs
R =			
H*	128	High	High
Me*	16	10	High
Et	4	2	3
n Pr	6	5	8
n-Bu	9	10	12
n Pentyl	4	4	11
Iso-Pr	1	1	1
Iso-Bu	20	13	30
sec-Bu	8	2	7
tert Bu	8	1	2
cyclo-Pentyl	0 5	4	19
cyclo-Hexyl	8	30	43

* These compounds were tested as the (−) isomers and the results have been calculated assuming that only half the material is biologically active.

Lands and Tanter (1953)

analogue, *Win* 5591 (IX 5), these all appear to be less active than Isoprenaline itself (Table IX 3, review by Lands and Tanter, 1953) Results on some other tissues are shown in Table IX 4 These indicate that activity at some receptors,

HO—⟨benzene ring⟩—CHOH—CH₂N⟨H, cyclopentyl⟩
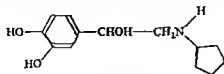
Win 5591, IX.5

those of the α-type, is maximal in adrenaline and *nor*adrenaline and that the replacement by methyl groups of both hydrogen atoms on the nitrogen of *nor*adrenaline reduces activity at both types of receptor

The results of Koopman (1960) and Ariëns (1960) show that, with these compounds, increase in the size of the alkyl group leads to increased affinity for the α-type receptors of the rat *vas deferens*, but also to decreased efficacy (Table IX 5) Some of the compounds, e g the N-2-phenyl*tert*butyl derivative

TABLE IX.4

Properties of N-Substituted Derivatives of (±)-norAdrenaline

HO—⟨benzene ring⟩—CHOHCH₂NRR'
HO—
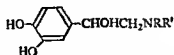
	Equipotent molar ratios relative to (~)-adrenaline			
NRR' =	Rat uterus	Rat colon	Rabbit ear	Cat nictitating membrane
NH₂ (−) .	75–300	0 2–1	1–3	1
NHMe (−) .	1	1	1	1
NHEt (±) .	0 5–1	1	12	45
NH*iso*Pr (±) .	0 5–1	1	10,000	Inhibits
NMe₂ (±) .	850	60	40	25

Gaddum, Peart, and Vogt (1949)

TABLE IX.5

Properties of N-Substituted Derivatives of (±)-norAdrenaline

HO—⟨benzene ring⟩—C(H)(OH)—CH₂NHR
HO—
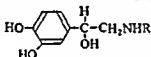
	On isolated rat *vas deferens*			On calf tracheal muscle
R =	Equipotent molar ratio relative to (±)-adrenaline	'Intrinsic activity' (page 7)	pA₂ for antagonism	Equipotent molar ratio relative to (±)-adrenaline
H* . . .	3	1	—	8
Me* . . .	1	1	—	1
Et . . .	2–4†	0 9	—	—
n-Pr . . .	100	0 3	—	—
*iso*Pr . . .	6	0 4	—	0 08
—CMe₃ . .	—	—	—	0 06
—CMe₂CMe₃ .	—	—	3 6	—
—CHMeCH₂Ph .	—	—	5 0	—
—CMe₂CH₂Ph .	—	—	5 5	0 02
—CHMeCH₂CH₂Ph.	—	—	5 2	0 025
—CMe₂(CH₂)₃Ph .	—	—	5 1	—

* These compounds were tested as (~) isomers, and the results have been calculated assuming that only half the material is biologically active.
† Ariëns gives the pD₂ as 5 0, but Koopman gives 4 7.

Ariëns (1960); Koopman (1960).

(IX 6), had considerable antagonist activity, but were, nevertheless, powerful agonists on the receptors of the β-type in the calf tracheal muscle

$$HO-\text{(ring)}-CHOH-CH_2-N\overset{H}{\underset{Me}{|}}-\overset{Me}{\underset{Me}{C}}-CH_2Ph$$
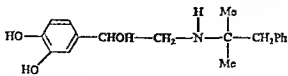
IX.6

Stereospecificity of Isoprenaline

Isoprenaline had been resolved and the isomers obtained in a very high degree of purity by Lands, Luduena, and Tullar (1954) as with adrenaline and noradrenaline, it is the (—)-isomer which is active, the equipotent molar ratio of the (+)-isomer relative to the (—)-isomer was found to be 600–1,600 on the cat blood pressure, 300–600 on dog blood pressure, 200–800 on the intact cat uterus, and 800–1,600 on isolated rat uterus On perfused guinea pig lungs, the ratio was 800 (Luduena, Von Euler, Tullar, and Lands, 1957) Dornhorst and Herxheimer (1958) found the ratio to be 50 in experiments in man These results suggest an even higher degree of stereospecificity than is observed with adrenaline and noradrenaline (Table IX 2), which is consistent with the idea that the isopropyl group contributes greatly to the fit of the molecule at the β-receptors If the equipotent molar ratio indicates only the difference in affinity (i e assuming the efficacy of the isomers to be the same, which may very well be untrue), a ratio of 100 implies that there is a difference of 2 8 Kcals in the free energy of adsorption of the two forms This would be consistent with the formation of a hydrogen bond by the alcoholic hydroxyl group with the receptor The apparently greater stereospecificity of Isoprenaline compared with adrenaline or noradrenaline suggests that the isopropyl group might, in the (+) isomer, interfere sterically with the attachment of the molecule If these (+)-isomers only are considered, activity at β-receptors may actually decline, as the size of the substituent is increased beyond the point where it is bulky enough to interfere in this way, on the perfused guinea pig lungs (+)-adrenaline was four times as active as (+) Isoprenaline

Effects of Substitution on the Carbon Atom Adjacent to the Nitrogen Atom

The introduction of substituents α- to the nitrogen atom produces an additional asymmetric centre, but the catecholamines of this type which have been studied so far appear usually to have been tested as racemates The α substituent also reduces the susceptibility of the molecule to attack by amine oxidases (review, Blaschko, 1952), the reaction must proceed through the substituted anil, —CR=NH, and occurs much less readily Compounds of this type may inhibit the oxidation of other amines by these enzymes This inhibitory property could account for some of the pharmacological effects of the compounds if adrenaline and noradrenaline really are inactivated by amine oxidases to any extent. Such a mechanism has been invoked by Gaddum and Kwiatkowski (1938) to explain the action of the α methyl derivative,

ephedrine (page 308), but may only be true with certain drugs acting on certain tissues For reasons discussed below, it seems unlikely to account to any extent for the properties of the substituted catecholamines

The compounds α-methylnoradrenaline (*Corbasil*, IX 7) and α-methyladrenaline (dihydroxyephedrine, IX 8) produce effects on blood-pressure which qualitatively resemble those of adrenaline, Cameron and Tainter (1936)

$$HO-\!\!\!\!\bigcirc\!\!\!\!-CHOH-\overset{R'}{\underset{}{CH}}-NHR$$

R = H, R' = Me, IX.7,
R = Me R' = Me, *Dihydroxyephedrine*, IX 8,
R = H, R = Et, α Ethylnoradrenaline, IX 9
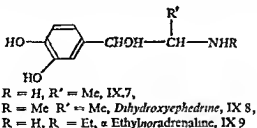
obtained equipotent molar ratios relative to (−) adrenaline of 12 for the former and 41 for the latter in experiments with cats Gaddum, Peart, and Vogt (1949) obtained ratios for α-methylnoradrenaline of 10 on the rat uterus, 5 on rat colon, 1,000 to 5,000 on the rabbit ear, and 200 on the cat nictitating membrane Schaumann (1936) found that a (+) isomer of the compound had only 1/160th of the activity of the corresponding (−)-isomer on the blood-pressure (animal not stated) Luduena, Von Euler, Tullar, and Lands (1957) found the (+) isomer inactive in perfused guinea-pig lungs, and Vane (1960) has found it only feebly active on the rat fundus strip

The substitution of an α-methyl group, accordingly, reduces activity at the α-receptors The compound α-ethylnoradrenaline (IX 9) was found by Cameron and Tainter (1936) to produce a fall in blood-pressure, and α-ethyl derivatives of Isoprenaline, N-*cyclo*pentyl, and N-*cyclo*hexyl noradrenaline have been found to have high depressor and bronchodilator activity (Table IX 6) It is remarkable how activity varies with the size of the α-substituent, being greatest in the unsubstituted and α-ethyl compounds, the α methyl, α n propyl and α-*iso*-propyl derivatives appear to be very much less active

Because α-substituents reduce activity at α-receptors it would seem most unlikely that the pharmacological properties of the compounds depend to any extent on their ability to block amine oxidases This contention is further supported by the way in which activity on the bronchi runs in parallel with activity in lowering the blood pressure If the dilatation of the bronchi were to any extent due to potentiation of the transmitters, the compounds should be pressor rather than depressor. The high stereospecificity of α-methyladrenaline, too, is scarcely in accord with an action dependent upon inhibition of amine oxidases, these enzymes not being particularly stereospecific (page 338)

Effects of Removal of the Alcoholic Hydroxyl Group

Analogues of adrenaline and noradrenaline lacking the alcoholic hydroxyl group were studied by Barger and Dale (1910) and found to be much less

TABLE IX 6

Activity of C-Substituted Derivatives of (±) norAdrenaline
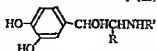
$$HO-\underset{HO}{\bigcirc}-CHOHCHNHR'$$

	Equipotent Molar ratios relative to (±)-Isoprenaline	
	Dilatation of bronchi, guinea pigs	Fall in blood pressure, anaesthetized dogs
R' = H		
R = H (noradrenaline)	150	Pressor
Me (*Corbasil*)	100	Pressor
Et (*Butanephrine*)	100	200
Pr	1,000	Pressor
R' = isoPr		
R = H (Isoprenaline)	1	t
Me	1,000	80
Et	2 5	16
Pr	>1 000	>3,000
isoPr	1,000	>3 000
R' = cyclopentyl		
R = H	0 5-0 7	3
Me	>1,000	50
Et	1	14
Pr	1,000	2,000
isoPr	>1,000	Slightly pressor
R = cyclohexyl		
R = H	10	30
Me	100	500
Et	7	500
Pr	>1,000	400

Lands and Tainter (1953)

active Pressor activity was greatest in the analogue of adrenaline and de-clined with increasing size of the alkyl group (Table IX 7) The first member of the series (3 : 4-dihydroxyphenylethylamine, *Dopamine*, *Hydroxytyramine*, IX 1) is of interest because it is now known to be the precursor of noradren-aline, being hydroxylated by 'dopamine hydroxylase' (Kirshner, 1960) This compound, however, is less active than the analogue of adrenaline (*Epinine* IX. 10) *even in tests, such as the cat blood pressure*, where noradrenaline appears to be more active than adrenaline In the experiments of Barger and Dale (1910) the equipotent molar ratio of (±)-noradrenaline relative to (±)-adrenaline was 0 70 and in the experiments on dogs the ratio for the (—)-isomers was 0 65

The activity of the compounds on β-receptors is also low Cameron and

TABLE IX 7

Pressor Activity of Catechol Ethylamines

$$HO-\text{(ring)}-CH_2CH_2NHR$$
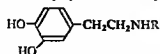
	Equipotent molar ratios relative to (−)-adrenaline		
R =	Cats		Dogs
	(a)	(b)	
H (*Dopamine*, IX 1)	70	65	50
Me (*Epinine*, IX 10)	14	12	11
Et	46	—	—
n Pr (IX 11)	280	—	—
*iso*Pr (IX 11)	—	—	Depressor
(−)-*nor*Adrenaline	0 70	—	0 65

Results for cats in column (a) were obtained by *Barger and Dale (1910)*, the compounds were compared with (±)-adrenaline and the figures have been calculated on the assumption that only half this material is biologically active. Results in column (b) were obtained by *Tainter (1930)* and *Cameron and Tainter (1936)* The result for *Dopamine* in dogs was obtained by *Alles (1933)* and the other results by *Lands et al (1948)*

Tainter (1936) obtained an equipotent molar ratio for *Epinine* relative to (−) adrenaline of 50 in the guinea pig perfused lungs, and Lands *et al* (1947, 1948) have commented on the very feeble activity of the *iso*propyl compound (IX 11) compared with Isoprenaline They state that 'in the absence of an

$$HO-\text{(ring)}-CH_2CH_2NHR$$

R = Me *Epinine*, IX 10
R = *iso*Pr, IX 11
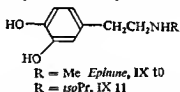
hydroxyl on the β carbon atom of the side chain the N-*iso*propyl group does not appear to increase bronchodilating action', but a strict quantitative comparison of the compounds in this series has not been made Vane (1960) has found that *Dopamine* and *Epinine* have only feeble activity on the receptors of the rat fundus strip, the equipotent molar ratios relative to (−) adrenaline being 250 and 33 respectively
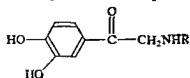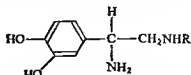
IX 12   IX 13

Analogous β keto compounds (IX 12, Barger and Dale, 1910, Lands *et al*, (1948) and β amino compounds (IX 13, Lehmann and Randall, 1948) appear to have much the same activity as the catecholethylamines

Meta-hydroxy Compounds

The *m* hydroxy compound (*Neosynephrine*, *Metasympatol*, IX 14) analogous to adrenaline was found by Kuschinsky and Oberdisse (1931) and Tainter (1932) to be quite active, the estimates of the equipotent molar ratio relative to (—) adrenaline on the cat blood-pressure were 15 and 5·6 respectively Della Bella and Galli (1955) compared the activities of this compound, and of the analogue of *noradrenaline*, with those of adrenaline and noradrenaline

$$HO \diagdown \diagup\hspace{-0.5em}\bigcirc\hspace{-0.5em}\diagup\diagdown CHOH{-\!-}CH_2NHR$$

R = Me, *Neosynephrine*, *Metasympatol*,
IX 14,
R = H, IX.15,
R = isoPr, IX.16
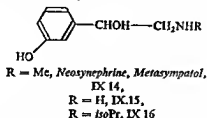
themselves on the blood-pressure of the anaesthetized dog They found that the dose-response curves all appeared to be parallel and obtained equipotent molar ratios relative to (±)-adrenaline of 0·6 for (±) noradrenaline, 6 for (±)-2 (*m* hydroxyphenyl)-2-hydroxyethylamine (IX 15), and 11 for (±)-2-(*m* - hydroxyphenyl) - 2 - hydroxyethylmethylamine (*Neosynephrine*) Lands (1952) obtained an equipotent molar ratio of 8 for the *m* hydroxy analogue of *noradrenaline* relative to (—)-adrenaline on the dog blood-pressure, and in similar tests with the optical isomers of *Neosynephrine* Lands, Nash, Granger, and Dertinger (1947) obtained ratios of 7 for the (—)-isomer, 12 for the racemate and 77 for the (+)-isomer

The analogue of Isoprenaline (*p*-desoxy-Isoprenaline, IX 16) is reported by Lands and Tainter (1953) to lower the blood-pressure in anaesthetized dogs, the equipotent molar ratio relative to (±)-Isoprenaline being 200–300, and in the perfused guinea-pig lung test the ratio was 80

With these *m*-hydroxy compounds the introduction of a methyl group α- to the amino group appears to reduce activity, as it does also but to a greater extent in the series of catecholamines Tainter (1932) obtained an equipotent molar ratio of 11·5 for the pressor activity in cats of (±)-3-(*m*-hydroxyphenyl)-3-hydroxy-2 aminopropane (IX·17) relative to (—) adrenaline,

$$HO \diagdown \diagup\hspace{-0.5em}\bigcirc\hspace{-0.5em}\diagup\diagdown CHOH{-\!-}\overset{\displaystyle Me}{\underset{\displaystyle}{CH}}{-\!-}NHR$$

R = H, IX.17,
R = Me, IX.18
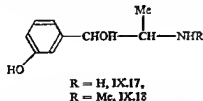
which indicates that the compound is less active than *Neosynephrine* and also, almost certainly, than the analogous *nor*-compound (IX 15, see above) Tainter, Peddeo, and James (1934) obtained equipotent molar ratios of 20 for (—)-Neosynephrine and 22 for (—)-3 (*m*-hydroxyphenyl)-3-hydroxy-2-methylaminopropane (IX 18) relative to (—) adrenaline in the guinea-pig lung test

With these *m*-hydroxy compounds, too, as with the catecholamines, removal of the alcoholic hydroxyl group reduces activity Barger and Dale (1910) observed that *m*-tyramine (IX · 19) had about the same pressor activity in cats as tyramine itself (IX 20), this being about one-hundredth of that of

$$HO\text{—}\underset{}{\bigcirc}\text{—CH}_2\text{CH}_2\text{NH}_2$$
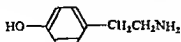
Tyramine, IX.20

adrenaline, and Lands and Tainter (1953) found that the equipotent molar ratio for the compound analogous to Isoprenaline (*m*-hydroxyphenylethyl*iso*-propylamine; IX 21) relative to (±)-Isoprenaline in lowering the dog blood-pressure was about 800

$$\underset{HO}{\bigcirc}\text{—CH}_2\text{CH}_2\text{NHR}$$
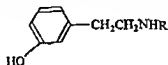
R = H, *m*-tyramine, IX 19,
R = *iso*Pr, IX 21

In this series of compounds lacking the 4-hydroxyl group, therefore, the relationships between structure and activity seem to be very similar to those for the catecholamines, but at all types of receptor, though the actions of the compounds are apparently the same as those of the catecholamines, the activity is considerably lower.

Para-hydroxy Compounds

The *p*-hydroxy compound analogous to adrenaline (*Synephrine, Parasympatol*, IX 22) is only feebly active, Kuschinsky (1930) obtained an equipotent molar ratio relative to (−) adrenaline for pressor activity in cats of 60 to 100 for the (±) form, and Tainter and Seidenfeld (1930) obtained a ratio of 58 for the (−)-isomer Lands and Grant (1952) found the ratio to be as high as 310 for

$$HO\text{—}\bigcirc\text{—CHOH}\text{—CH}_2\text{NHR}$$
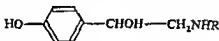
R = Me, *Synephrine, Parasympatol*, IX.22,
R = H, IX 23,
R = *iso*Pr, IX.24

the racemate in dogs Both Kuschinsky (1930) and Tainter and Seidenfeld (1930) observed that the (−) isomer was about twice as active as the racemate and the (+) isomer only feebly active Pratesi, La Manna, Campiglio, and Ghislandi (1958) have shown that the (−)-isomer has the R configuration, like (−) adrenaline The *p*-hydroxy analogue of *nor*adrenaline (IX 23) appeared to be slightly more active, Lands and Grant (1952) obtained an equipotent molar ratio relative to (−) adrenaline of 85

An increase in the size of the N-alkyl group appeared to reduce pressor activity and to increase depressor and bronchodilator properties Tainter and

Seidenfeld (1930) observed that *Synephrine* did not cause significant broncho-dilatation, but Lands and Tainter (1953) found that for the isopropyl analogue (IX.24) the equipotent molar ratio for the racemate relative to (±) Iso prenaline was 200 for depressor activity in dogs and 400 in the perfused guinea-pig lung test The compounds, accordingly, have some activity, but are only feeble, being weaker even than the analogous m-compounds Ariens (1960) has shown that in a series of N-substituted derivatives of *Synephrine* an increase in the size of the substituent decreased the activity on the isolated *vas deferens* of the rat, but still higher homologues were quite potent antagonists (Table IX 8) Two of these, *Isoxsuprine* (*Duvadilan*, IX 25) and *Nylidrine*

HO—⟨ ⟩—CHOH—C—NHR
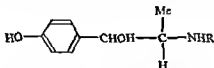
R = —CHMe—CH₂OPh *Isoxsuprine*, IX 25
R = —CHMe·CH₂Ph, *Nylidrine*, IX.26
R = H, IX.27,
R = Me, *p* hydroxyephedrine IX.29

(IX 26), which also contain a methyl group α to the nitrogen atom, are used for producing vasodilatation This additional action may be due, at least in part, to the compounds retaining stimulant activity at β adrenergic receptors (Lish, Dungan, and Peters, 1960), even though they block α receptors

The introduction of a methyl group α- to the amino group does not appear to alter the intensity of the activity greatly, Tainter (1932) obtained an equipotent molar ratio for (±) 3 (p-hydroxyphenyl) 3 hydroxy 2 aminopropane (IX 27) relative to (—)-adrenaline of 67 for pressor activity in cats The re moval of the alcoholic hydroxyl group also does not markedly effect activity

TABLE IX 8

Activity of α Substituted Derivatives of Synephrine on the Isolated vas de ferens *of the Rat*

HO—⟨ ⟩—CHOHCHRNHR
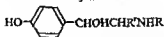
R	R	Agonist activity Equipotent molar ratio relative to *Synephrine*	Antagonist activity pA₂
H	H	0·3	—
Me	H (*Synephrine*)	1 0	—
n Pr	H	13	—
tert Bu	H	40	—
—CMe₂CH₂Ph	H	—	5 6
—CHMe(CH₃)₂-Ph	H	—	5 5
—CHMe(CH₃)₂Ph	Me (*Nylidrine*)	—	6 2
—CHMeCH₂OPh	Me (*Isoxsuprine*)	—	6 0

Ariens (1960)

The results of Barger and Dale (1910) indicate that tyramine and N-methyl-tyramine (IX 20 and 28) have activity of the same order as that of *Synephrine* and *p-hydroxyephedrine* (*Suprifene*, IX 29) The equipotent molar ratios relative to (−)-adrenaline appear to be between 100 and 200, but estimates vary

$$HO\!\!-\!\!\langle\text{ring}\rangle\!\!-\!\!CH_2CH_2\!\!-\!\!NHMe$$
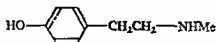
IX 28

greatly (Bovet and Bovet-Nitti, 1948) Desoxy compounds with an α-methyl group appear to be more active, Mügge (1937) obtained an equipotent molar ratio for *Pholedrine* (*Veritol*, IX 30) relative to (−)-adrenaline of between 10 and 50 on the cat blood-pressure and Alles (1933) a ratio of between 50 and

$$HO\!\!-\!\!\langle\text{ring}\rangle\!\!-\!\!CH_2\overset{Me}{\underset{}{CH}}\!\!-\!\!NHR$$
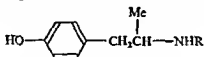
R = Me, *Pholedrine*, IX.30,
R = H, *p-Hydroxy amphetamine*, IX.31

100 for *p-hydroxyamphetamine* (*Paredrine*, IX 31) in dogs The responses produced by all these drugs, however, are different in character from those produced by adrenaline In particular the effects last much longer, making quantitative estimation of activity intrinsically impossible and accounting for the great variation in the results

Cameron and Tainter (1936) considered that the actions of some of these compounds resembled those of ephedrine and other compounds without phenolic groups (see below) rather than those of adrenaline and noradrenaline Moreover, even some compounds which produce a transient response, such as tyramine (Fig IX 6), do not appear to be acting exactly like adrenaline and
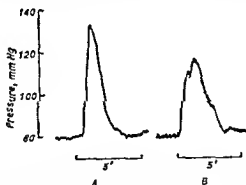
FIG IX 6 *Blood pressure responses in the anaesthetized rat to*
A 27 nMoles of (−)-adrenaline and B 20 μMoles of tyramine Note the difference in the time course of the response

noradrenaline The effects of adrenaline on blood-vessels are potentiated by cocaine (possible reasons for this are discussed on page 340), and Morton and Tainter (1940) tested the effects of cocaine on the responses of the perfused cat's hind limb to a wide variety of compounds, they also observed the effects on activity of changing the perfusion fluid from physiological saline to blood

The results (Table IX 9) show distinct differences in the behaviour of the compounds, these being in general related to differences in chemical structure The catecholamines all behaved similarly in being potentiated by cocaine and about as active in the presence of blood as in the presence of physiological saline The *m* hydroxy compounds behaved in more or less the same way, but most of the *p*-compounds and all the non phenolic compounds were antagonized by cocaine and much more active in the presence of blood It seems unlikely, therefore, that these latter compounds are acting in the same way as adrenaline

Carlsson, Rosengren Bertler, and Nilsson (1957) found that tyramine did not raise the blood pressure of animals which had been treated with reserpine, but Burn and Rand (1958) observed that pressor activity returned after the animal had been given *noradrenaline* This was interpreted as indicating that tyramine acted by causing a release of transmitter from the local stores, which were depleted when the animal was treated with reserpine and which became replenished after the animal had been given some noradrenaline (by slow intravenous infusion) Burn and Rand (1960) subsequently found that although the sensitivity to tyramine could be restored by 3 4-dihydroxy phenylethylamine (dopamine), or the amino acids, 3 4-dihydroxyphenyl alnine, *m* tyrosine, or even phenylalanine, it was not restored by adrenaline

These findings could explain why the relationships between structure and activity in this series of *p*-hydroxy compounds are different from those in the *m* hydroxy or 3 4-dihydroxy series Compounds, such as *Synephrine* (IX 22) or 2 (*p*-hydroxphenyl) 2 hydroxyethylisopropylamine (IX 24) which closely resemble adrenaline and Isoprenaline, have some feeble activity on the adrenergic receptors even though they lack the *m* hydroxyl group Compounds without the alcoholic group, such as tyramine (IX 20) N methyltyramine (IX 28) *Pholedrine* (IX 30) and *p hydroxyamphetamine* (IX 31) appear to be more active as pressor agents than would be expected because they act mostly by a different mechanism, *Pholedrine*, in particular may be acting like ephedrine (see below) as well as like tyramine On the β-receptors in the guinea pig lung however, these compounds without the alcoholic hydroxyl group are inactive (Lands and Tainter, 1953) which suggests that the storage mechanism at these sites may be different from that at sites containing α receptors

Compounds without Phenolic Groups

Phenylethylamine Derivatives

Barger and Dale (1910) studied some aromatic amines without a phenolic hydroxyl group and found that 2 phenyl 2 hydroxyethylmethylamine (IX 32)

Ph–CHOH–CH₂–NHR   Ph–CH₂–CH₂–NHR
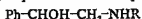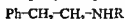
R = Me IX 32   R = Me IX 34

R = H IX 33   R = H IX 35

2 phenyl 2 hydroxyethylamine (IX 33), 2 phenylethylmethylamine (IX.34), and 2 phenylethylamine (IX 35) all had weak pressor activity of about the same order of magnitude Results obtained by Lands and Grant (1952, Table

TABLE IX 9

Vasoconstrictor Properties of Substances on Perfused Hind Limb of the Cat

	Equipotent molar ratio* relative to (—)-adrenaline in preparation perfused with		Ratio	Effect of cocaine
	Locke's solution	Defibrinated blood		
HO—⬡—OH				
(±) CHOHCH₂NH₂ (norAdrenaline)	2 0	1 2	1 7	S
(±) CHOHCHMeNH₂ (Corbasil)	99	3 2	3 1	S
(±) CHOHCHMeNHMe	20	29	0 7	S
CH₂CH₂NHMe (Epinine)	6 7	7 9	0 85	S
HO—⬡				
(—) CHOHCH₂NHMe (Metasympatol)	2 5	1 2	2 0	S
(±) CHOHCHMeNH₂	2 10	5 3	4 0	No change
(—) CHOHCHMeNHMe	2 90	1 50	2 0	—
HO—⬡				
CH₂CH₂NH₂ (Tyramine)	6,700	3 9	174	D
(±) CH₂CHMeNH₂	8 000	3 7	214	—
⬡				
(—) CHOHCHMeNHMe (Ephedrine)	34 000	80	430	D
(±) CHOHCH₂NH₂	99	4 9	20	D
(±) CHOHCHMeNH₂	40,000	44	890	D
CH₂CH₂NH₂	100	9 2	11	D
(±) CH₂CHMeNH₂ (Amphetamine)	81,000	Dilator		D

* Calculated on the assumption that the original figures refer to weights of base the optical activity of the material tested is also not always specified, the sign indicated here is that which seems most likely from what is known about the material available when this work was done Errors in these figures will not affect the ratio, which is the most important feature of the table If this is greater than unity the compound is more active in the presence of blood

S indicates increased sensitivity, D indicates diminished sensitivity, — indicates not tested

Note — The log dose–response curve for adrenaline in the preparation perfused with Locke's solution was not greatly different from the curve for the preparation perfused with blood

Morton and Tainter (1940)

TABLE IX 10

Pressor Activity of Phenylethylamine Derivatives in Anaesthetized Dogs

PhCHRCH₂NHR'

R	R'	Equipotent molar ratio relative to (−)-adrenaline
H	H	100
H	Me	117
OH	Me	244

Lands and Grant (1952)

IX.10) indicate that the compounds actually differ slightly in activity, those with β-hydroxyl or N methyl groups being weaker than the simple phenylethylamine They all produce effects which last much longer than those of adrenaline (Fig IX 6) and consequently estimates of activity relative to adrenaline are meaningless and very variable (Bovet and Bovet Nitti, 1948), estimates of activity relative to each other, however, may be more informative The substitution of a methyl group α- to the amino group, as in the compounds ephedrine (IX 36) and Amphetamine (*Benzedrine*, IX 37), appears to prolong

$$\text{PH-CHR'-CH-NHR}$$
$$\overset{\text{Me}}{|}$$

R = OH, R = Me Ephedrine IX.36
R = H, R = H Amphetamine, IX 37,
R = H, R = Me *Methylamphetamine*, IX.38
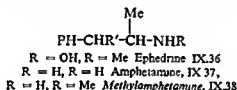
their actions even further, although it does not greatly affect their activity as assessed by the dose required to produce a particular rise in pressure These substances are particularly interesting, partly because their action is sustained and therefore potentially useful, partly because they are absorbed when given by mouth (unlike adrenaline), and partly because they stimulate the central nervous system

Ephedrine is an alkaloid obtained from *Ma Huang*, a drug which has been in use in Asia for several thousand years It was isolated by Nagai (1887) but its pharmacological properties were first studied by Chen and Schmidt (1925) It contains two asymmetric centres and there are, therefore, four isomers, (+)- and (−) ephedrine and (+)- and (−) ψ ephedrine Emde (1929) showed that (−)-ephedrine and (+)-ψ-ephedrine could both be reduced to (+)-des oxyephedrine (Fig IX 7) and so must have the same configuration at the carbon atom α to the amino group Freudenberg and Nikolai (1934) showed that this configuration was the same as that of (+)-alanine and that in (−)-ephedrine the arrangement at the carbon atom β- to the amino group was the same as in (−) mandelic acid The absolute configuration of (−)-ephedrine must, therefore, be 3R (3 phenyl 3 hydroxy)-2S (methylamino)propane, and (+)-ψ-ephedrine will have the 3S-2S structure This has been confirmed by

crystallographic analysis (Phillips, 1954) and by nuclear magnetic resonance studies on cyclic derivatives (Fig IX 7, Hyne, 1959)

The activity of the various isomers of ephedrine has been studied by Chen, Wu, and Henriksen (1929) and the results (Table IX 11) indicate that although the (−) isomer of ephedrine is the most active, the differences between the various forms are not as great as with the isomers of adrenaline and noradren-

Cyclic derivatives of (−) ephedrine (on left) and (±) ψ ephedrine (on right) studied by Hyne 1959
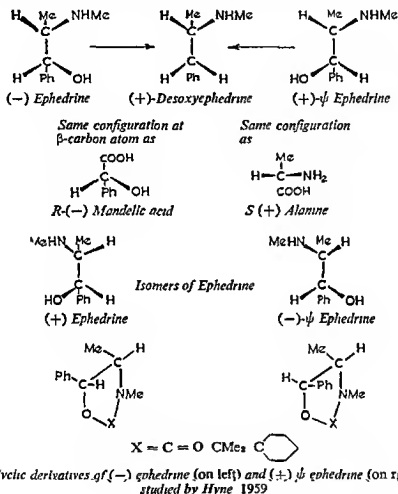
FIG IX 7

aline (Table IX 2) This is further support for the idea that ephedrine does not act in the same way as adrenaline Even though (−) ephedrine has the same configuration at the β carbon atom as (−) adrenaline (Fig IX 5), the relationships between configuration and activity are difficult to interpret If the (−) isomer is attached through the alcoholic group why is not the (−) ψ isomer the next most active for it has the same configuration at the β-carbon atom? If this is because the arrangement at the α-carbon is different, why is not the (+) isomer the least active for it has the same configuration at the α carbon atom as (−) ψ ephedrine and the 'wrong' configuration at the β carbon atom?

A possible explanation may be that there is restricted rotation about the αβ C–C-bond with the bulky methyl and amino groups placed away from the benzene ring (Fig. IX 8) In the isomers of ephedrine, this would lead to the amino and hydroxyl groups being *trans* to each other, whereas in the isomers of ψ-methyl ephedrine they would be *cis* Prelog and Häfliger (1950) have obtained evidence that this occurs in solution: (–)-ephedrine is a slightly weaker base than (+)-ψ-ephedrine (the pK$_a$ values at 22° are 9 58 and 9 74 respectively) and (–)-N-methylephedrine is a distinctly weaker base than (+)-N-

TABLE IX 11

Pressor Activity of Isomers of Ephedrine

	Equipotent molar ratios relative to (–)-ephedrine in anaesthetized cats
(–)-Ephedrine	1 0
(±)-Ephedrine	1 3
(+)-Ephedrine	2 9
(+)-ψ Ephedrine	5 2
(±)-ψ Ephedrine	8 8
(–)-ψ Ephedrine	35 0

Chen, Wu, and Henriksen (1929)

methyl-ψ-ephedrine (the pK$_a$ values at 26° are 9 22 and 10 02 respectively) It is possible that the ionized form would be stabilized by hydrogen bond formation if there is a hydroxyl group close enough (a solvent molecule is also involved), and hence it is argued that in the stronger base the amino and hydroxyl groups must be *cis* (see also Brown, McDaniel, and Häfliger, 1955) Accordingly, it is possible that when absorbed at the receptor, the hydroxyl group, amino group, and benzene ring in (–)-ephedrine lie almost in the same plane, and that the difference between the (–) and (+) isomers arises from the α-methyl group obstructing the fit of the (+)-isomer (Fig IX 8) In (+)-ψ-ephedrine, the amino and hydroxyl groups can less readily assume a *trans* configuration, similar to that shown for (–)-ephedrine, and the methyl group is then in a position similar to that in (+)-ephedrine, in which it might obstruct attachment to the receptor, to achieve a similar arrangement of the hydroxyl group, amino group, and benzene rings in (–)-ψ-ephedrine, the methyl group would have to be in exactly the opposite position, and it would be greatly hindered from doing this by the benzene ring It must, on the other hand, be pointed out that, although the results of Phillips (1954) confirm the absolute configuration of (–)-ephedrine, they show that, in the crystalline hydrochloride, the hydroxyl and methylamino groups are not *trans* but *cis* The relative positions of these groups and the chloride ions, however, indicate appreciable interaction and the gain in energy from this could account for the assumption, in the crystal, of what would be, in solution, a less stable configuration

This explanation supposes that the hydroxyl group, amino group, and the benzene ring are all involved in the attachment at the receptors. It may be questioned whether the hydroxyl group can be. In the phenylethylamine derivatives without the *α* methyl group the presence of a β-hydroxyl group does not appear to effect activity and in the tyramine series it appears actually to decrease it. With these non-phenolic *α* methyl compounds there is no convincing evidence that a β-hydroxyl group either increases or decreases activity. Tainter (1933) found that norephedrine (presumably a mixture of the isomers) was considerably more active on the blood pressure than Amphetamine, but
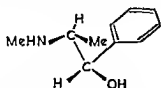
Preferred configuration of (−)-ephedrine? HO– trans to NHMe

Preferred configuration of (+)-ψ-ephedrine? HO– cis to NHMe
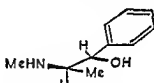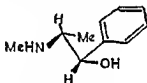
Hypothetical fit of (−)-ephedrine (on left) and (+)-ephedrine (on right) to receptor

Hypothetical fit of (+) ψ ephedrine (on left) and (−) ψ ephedrine (on right) to the same receptor, note that in the right hand picture the methyl group on the carbon atom α- to the amino group is forced in the direction of the benzene ring, with which it may interfere sterically

FIG IX 8

there does not appear to be much difference between the activities of (−)-ephedrine and *methylamphetamine* (desoxyephedrine, *Methedrine, Pervitine*, IX 38, see, for example, Domenjoz and Fleisch, 1940)

The desoxy compounds, however, are much less stereospecific. Swanson, Scott, Lee, and Chen (1943) found that, on the blood pressure of the spinal dog, the (−)-isomers of Amphetamine and *methylamphetamine* were more active than the (+)-isomers, but the equipotent molar ratio for the (+)- relative to the (−)- was very low, being 1 4 for both Amphetamine and *methylamphetamine*. In anaesthetized dogs, however, Lands, Nash, Granger, and Dertinger (1947) found the (+) isomer of *methylamphetamine* to be more active than the (−)-, the ratio being 2 3 1. These results indicate that there is

L

little difference between the activities of the isomers and consequently the stereospecificity of ephedrine must arise from the presence of the hydroxyl group

The isomers of *norephedrine* have not been studied in the same detail as those of ephedrine, but the results which are available (reviewed by Chen and Schmidt, 1930) indicate that (+)-ψ norephedrine is more active than (−)-ψ norephedrine These *nor*-compounds appear to be more active than the corresponding N methyl derivatives, and in this respect the phenylethylamine series resembles those already discussed

Other Amines

Several derivatives of *cyclo*Hexylethylamine were studied by Gunn and Gurd (1940), and Lands and Grant (1952) compared quantitatively the activity of a number of *cyclo*hexyl analogues with that of the analogues containing a benzene ring The former were consistently less active than the latter (Table IX 12) and likewise 4-hydroxy*cyclo*hexyl compounds were consistently less active than the analogous tyramine derivatives

TABLE IX 12

Pressor Properties of cycloHexylethylamine Derivatives in Anaesthetized Dogs

$$\bigcirc\!\!-\!CHR\!\cdot\!CH_2\!\cdot\!NHR$$
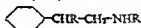
		Equipotent molar ratio relative to (−)-adrenaline	
		*cyclo*Hexyl compound	Phenyl analogue (Table IX.11)
R =	R =		
H	H	244	100
H	Me	160	117
OH	Me	900	244

Lands and Grant (1952)

Barger and Dale (1910) examined many aliphatic amines and found weak pressor activity in long chain compounds such as 2 aminoheptane (IX.39) and 2 amino-octane (IX 40) Many compounds of this type have been tested by

$$CH_3\!\cdot\!CH\!\cdot\!(CH_2)_n\!\cdot\!CH_3$$
$$\underset{NH_2}{|}$$
$$n = 4 \text{ IX 39,}$$
$$n = 5 \text{ IX.40}$$

$$\underset{H'}{\overset{H}{CH_3\!\cdot\!\underset{|}{C}\!\cdot\!CH_2\!\cdot\!\underset{|}{\overset{Me}{C}}\!\cdot\!CH_2\!\cdot\!CH_3}}$$
$$\underset{H}{\overset{|}{N}}$$
$$R = Me \text{ IX.41,}$$
$$R = H \text{ IX.42}$$
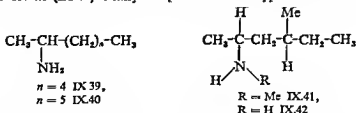
Swanson and Chen (1946) and by Marsh, Howard, and Herring (1951), and activity appears to be greatest in compounds such as 2 methylamino-4-methylhexane (IX 41), 2 amino-4-methylhexane (IX 42), 2 methylaminoheptane

(IX 43), and 2 aminoheptane (IX 39) These all contain the unit, –CHMeNHR, and may possibly be acting like Amphetamine, though less effectively

In both the *cyclohexyl* and simple aliphatic series of amines, there appears to be only a moderate degree of stereospecificity Lands, Nash, Granger, and Dertinger (1947) found (–)-N-methyl β *cyclohexylisopropylamine* (IX 44)

CH₃-C-(CH₂)₄-CH₃   [structure IX 43]   [structure IX 44]

$$CH_3\text{-}\underset{\underset{Me}{N}}{\overset{\overset{H}{|}}{C}}\text{-}(CH_2)_4\text{-}CH_3$$

IX 43

$$\text{cyclohexyl}\text{--}CH_2\text{--}\underset{CH_3}{\overset{Me}{CH}}\text{--}NHMe$$

IX 44
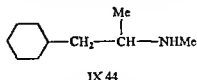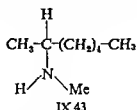
to be more active than the (+) isomer, the equipotent molar ratio for the latter relative to the former on the blood pressure of anaesthetized dogs being 2 5 In the same experiments, the (+) isomer of the benzene analogue, *Methylamphetamine*, was more active than the (–)-isomer (see above) The equipotent molar ratios relative to (–) adrenaline were 290 for the racemic form of the *cyclohexyl* compound and 200 for (±) *Methylamphetamine* For 2 aminoheptane in the spinal dog, Swanson, Steldt, and Chen (1945) found the (+)-isomer to be more active than the (–), the equipotent molar ratio for the latter relative to the former being 1 9 The equipotent molar ratio for the racemate relative to (–)-adrenaline was around 300

Benzene Derivatives with Substituents other than Hydroxyl

Alkylation of the phenolic groups in adrenaline and noradrenaline appears to reduce activity drastically The introduction of even one methyl group in the *m* position by the enzyme catechol O methyl transferase appears to reduce activity so much that this step is regarded by Axelrod, Weil Malherbe, and Tomchick (1959) as terminating the physiological effects of adrenaline and noradrenaline Bacq (1960) has challenged the use of the word 'terminating', because 3 O methyladrenaline (*Metanephrine*, IX 2, this compound should not be confused with *Neosynephrine*) appears to affect the sensitivity of tissues to adrenergic drugs although not producing responses itself The cat nictitating membrane, for instance, is more sensitive to sympathetic stimulation in the presence of 3 O methyladrenaline The activity of the compound, however, appears to be small, Kukovetz, Hess, Shanfield and Haugaard (1959) failed to obtain any effects on the rat heart with doses 1,000 times those of adrenaline and noradrenaline which were active The 2 5-dimethoxy compound, *Methoxamine* (IX 45), was also inactive This latter compound, however, caused a long lasting vasoconstriction and rise in blood pressure in doses about 100 times those of adrenaline and noradrenaline (Goldberg, Cotten, Darby, and Howell, 1953)

Alkylation of the phenolic groups in adrenaline should make the molecule less polar and so might greatly facilitate transport to the central nervous system Many compounds of this type, like the other non phenolic amines have marked stimulant activity on the central nervous system Mescaline (IX 46) is

an example These could produce changes in blood-pressure indirectly by stimulating the vasomotor centres (see page 85).

Singh Grewal (1952) found that 6-methyladrenaline (IX 47) produced responses very like those of adrenaline, but the compound was considerably less active, the equipotent molar ratio for the racemate relative to (—) adrenaline
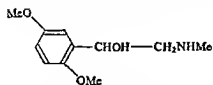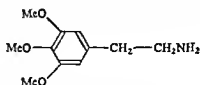
Methoxamine, IX.45   Mezcaline, IX 46

on the blood-pressure of the spinal cat being about 20 This compound was originally made because it could not be oxidized to an adrenochrome derivative It is also of interest because of the close proximity of the 6-methyl group to the alcoholic hydroxyl group which may, in consequence, be sterically hindered
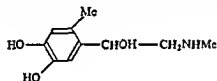
IX 47
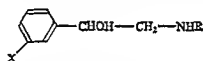
X = H₂N, IX 48,
X = F, IX.49

Lands (1952) studied a number of *m* amino analogues of adrenaline (IX 48) and found these to be considerably less active than the *m*-hydroxy analogues The analogue of noradrenaline was the most potent on the blood-pressure of anaesthetized dogs, but from its activity relative to (—)-adrenaline it appears to be only about one-tenth as active as the *m*-hydroxy compound (IX.15, page 302) Lands (1952) also studied a number of *m*-fluoro analogues (IX 49) and found these to be even weaker than the *m*-amino compounds
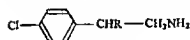
R = H, IX 50,
R = OH, IX 51
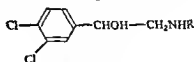
R = H, IX 52,
R = Me IX 53,
R = *iso*Pr, DCI IX 54

Tainter (1930) observed that *p*-chlorophenylethylamine (IX 50) was less active than phenylethylamine, and Hartung, Munch, and Crossley (1935) likewise found that the introduction of a *p*-chloro group into the corresponding alcohol (1-phenyl-1-hydroxy-2 aminoethane, IX 51) greatly reduced pressor activity The 3 4-dichloro compound (IX 52) analogous to noradrenaline was synthesized by Glynn and Linnell (1932) and also found to have only weak activity The equipotent molar ratio relative to (—)-adrenaline on the spinal cat appeared to be around 200 Powell and Slater (1958), however, found that the 3 4-dichloro analogues of noradrenaline, adrenaline,

and Isoprenaline were antagonists of the actions of adrenaline and Isoprenaline on tissues containing receptors of the β type, such as those in the blood vessels of the cat which dilate and lead to a fall in blood pressure, those in the bronchi of the dog and in the trachea of the guinea pig, and those in the rat uterus and rabbit intestine the effects on the heart were not so marked Ariens (1960) has obtained pA₂ values on the calf tracheal muscle of 4 0 for the analogue of noradrenaline (IX 52), 4 3 for the analogue of adrenaline (IX 53), and 4 9 for the analogue of Isoprenaline ('*DCI*', IX 54) The actions of the compounds may be complicated, however, by the weak activity at α-receptors and by the ability of *DCI* to act also as a non specific bronchodilator This non specific effect is more marked in *DCI* than in the lower homologues and can be detected by the ability to antagonize contractions of the preparation produced by barium ions, the action appears to resemble that of the alkaloid papaverine and does not involve the adrenergic receptors

Moran and Perkins (1958) found that *DCI* produced blockade of the adrenergic receptors in both intact and isolated mammalian hearts in doses which did not alter the responses to drugs which effect the heart by other mechanisms (digoxin, theophylline, and calcium chloride) The isopropyl compound was more active than the analogues of noradrenaline and adrenaline. Fleming and Hawkins (1960), however, observed that the action of *DCI* in the dog heart lung preparation is not that of a simple antagonist, stimulation precedes antagonism and the compound may well be really a partial agonist Ahlquist and Levy (1959) and Levy (1959) have shown that the actions of the compounds on the intestine are also more complicated than was first thought, the results have been interpreted by Furchgott (1960) as indicating the presence in the tissues of both α and β-types of receptor Claasen and Noach (1960) have found that *DCI* inhibits the action of adrenaline in stimulating glycogenolysis and raising the concentration of glucose in the blood

These 3 4-dichloro analogues of noradrenaline, adrenaline, and Isoprenaline are of considerable pharmacological interest because they may be used for investigating the types of adrenergic receptor present in the tissues Their actions are not so specific, however, that their use for this purpose always gives unambiguous results

Black and Stephenson (1962) have recently described the powerful blocking action at β adrenergic receptors of 2 isopropylamino-1 (2 naphthyl)ethanol (*Pronethalol, Alderlin*) This molecule is structurally very similar to *DCI*, the only difference being that at the 3- and 4- positions, a benzene ring is fused on to in *Pronethalol*, in place of the two chloro groups of *DCI* Unlike *DCI*, it is reported to have little agonist activity at α receptors Black and Stephenson found that the concentrations of *Pronethalol* which reduced the effects of adrenaline to half were 2×10^{-7} M on cat papillary muscle 8×10^{-9} M on guinea pig heart muscle (Langendorff's preparation) and 8×10^{-8} M on the guinea pig trachael chain In concentrations up to 4×10^{-5} M the compound was inactive against the effects of adrenaline on the rabbit uterus or perfused ear Dornhorst and Robinson (1962) showed

that the compound was also active in man, blocking the effects of Isoprenaline on the circulation Its action on the metabolic effects of adrenaline is not clear Pilkington, Lowe, Robinson and Titterington (1962) found that the compound blocked the rise in the level of free fatty acids, produced in response to adrenaline, whereas an α-blocking agent, *Phenoxybenzamine*, did not do this Neither compound, however, was effective in preventing the rise in the level of glucose produced by adrenaline

Relationships between Chemical Structure and Activity

The pressor properties of a number of compounds related to adrenaline are shown in Table IX 13 Although the figures are not based on a strict comparison of the compounds in one set of experiments, but were calculated by Gunn (1939) from results published by many authors using different preparations,

TABLE IX 13

Pressor Properties of Compounds Related to Adrenaline

	H H H C–C–N H H					Pressor effects[*]				Relaxation of rabbit intestine Equipotent molar ratio relative to adrenaline
						Equipotent molar ratio relative to adrenaline	Duration relative to adrenaline	pK_a 1	pK_a 2	
Phenylethylamine	H	H	H	H	H	50–130	3–4	—	9 86	>10 000
Amphetamine	H	H	H	Me	H	75–370	5–10	—	9 93	>10 000(±)
Phenylpropanolamine	H	H	OH	Me	H	50–250	7	—	9 44	10 000
Ephedrine	H	H	OH	Me	Me	90–180	7 10	—	9 49	2,000
Tyramine	HO	H	H	H	H	15–75	2	9 77	10 78	>10 000
Paredrinol	HO	H	H	Me	Me	27	10	9 53	—	—
O-Methyltyramine	MeO	H	H	H	H	250	4	—	—	—
Sympatol	HO	H	OH	H	Me	23 90	4	9 59	9 71	200(±)
Metasympatol	H	HO	OH	H	Me	4 6	2	9 61	9 70	10
Dopamine	HO	HO	H	H	H	29	1	8 92 8 87	10-63	50
Epinine	HO	HO	H	H	Me	11	2	8 86 8 90	10 61	1? 5
Corbasil	HO	MeO	OH	H	Me	4	2	8 85 8 73	9 75	8 5
norAdrenaline	HO	HO	OH	H	H	0 62	1	8 90 8 73	9 78	1-0
Adrenaline	HO	HO	OH	H	Me	1	2	8 88 8 71	9 90	1-0

[*] Results taken from the literature for experiments with a variety of mammalian preparations It appears that the figures for compounds with an asymmetric carbon atom were calculated for the racemates In the experiments with rabbit intestine the (–)-isomers were tested except with Amphetamine and Sympatol (as indicated)

† For the phenolic group these results were obtained by a spectrophotometric method and most were checked by potentiometric titration the pK_a values for the basic group were calculated from results obtained by potentiometric titrations after allowance for the effects of the phenolic group

Gunn (1939) Lewis (1954)

the Table forms a convenient summary and the results agree quite well with those obtained more recently (which have been discussed above) The dissociation constants of many of the compounds have been measured by Lewis (1954) and are included in the Table together with an indication of the properties of the compounds on isolated rabbit intestine Phenylethylamines and ethanolamines are strong bases, and those with phenolic groups are weak acids There is very little difference between the values for all the compounds the results for Isoprenaline, for instance (not included in the Table), are practically identical with those for adrenaline and indicate that, at physiological pH, the molecule is about 94 per cent present as the cation and only 5 per cent as the zwitterion It seems reasonable to expect that the former is the active species

For activity at α adrenergic receptors, three features appear to be necessary, a *m* phenolic group, an alcoholic hydroxyl group, and an amino group, in the same positions relative to each other as in (−) noradrenaline A *p* phenolic group appears also to be beneficial, but is not as essential as is the *m* phenolic group If the former only is present, as in tyramine, part of the activity of the molecule may be ascribed to an action on the mechanisms for storing noradrenaline, more than to an action on the adrenergic receptors themselves An increase in the size of the substituent on the nitrogen atom leads to a marked decline in activity, though this is an effect on efficacy, rather than affinity, because some of the compounds are antagonists The importance of the hydroxyl group for activity at the adrenergic receptor is shown by the high stereospecificity of all the active compounds The stereospecificity of the weaker compounds, however, is less marked and is consistent with the idea that these may be acting at sites other than the adrenergic receptors

The introduction of a methyl group in a position α- to the amino group appears to prolong the activity of a compound It was suggested by Blaschko, Richter, and Schlossman (1937) that adrenaline was destroyed by amine oxidase, and Gaddum and Kwiatkowski (1938) postulated that the actions of compounds of this type, such as ephedrine, might be due to inhibition of this destruction and consequent potentiation of the effects of adrenaline Noradrenaline is also destroyed by amine oxidase, but it is now clear that enzymes of this type are not primarily responsible for limiting the effects of either adrenaline or noradrenaline Moreover, substances are known which inhibit amine oxidases (see page 340), but have little activity on the blood pressure or on other preparations containing adrenergic receptors The actions of compounds such as ephedrine must, therefore, be ascribed to activity at the adrenergic receptors and/or on the storage mechanisms, and the prolonged activity brought about by the introduction of the α methyl group must be ascribed to the increased resistance of the compound itself to amine oxidases It is interesting that the effects of the substitution of an α methyl group on duration of action are greater in the phenylethylamine and tyramine series than in the *m* hydroxy- or 3 4 dihydroxy- phenylethylamine series In the latter, the compounds would be expected to be inactivated by O methylation, whereas the former would be expected to be destroyed by amine oxidases

For activity on β-adrenergic receptors the requirements appear to be rather similar, a m-phenolic group, an alcoholic group, and an amino group, but these substituents differ from the α-receptors in that a large substituent, such as isopropyl, on the nitrogen atom, which would destroy efficacy at the α-receptors, increases activity at the β-receptors The effects of altering the alcoholic hydroxyl group and of introducing an α-methyl group on activity at β-receptors appear to be very similar to the effects on activity at α-receptors The effects of substitution in the benzene ring, however, are different The 3 4-dichloro compounds discussed above block the β-receptors, but retain weak stimulant activity at α-receptors

It is interesting that quaternary derivatives are inactive at both types of receptor This might indicate that the drugs have to pass through some membrane in order to reach the receptor and that this membrane is impermeable to quaternary salts which are permanent cations On the other hand, all the actions of adrenaline are relatively rapid in onset, and it is conceivable that the quaternary compounds fail to act simply because of the bulk of the groups around the nitrogen atom At α-receptors, for example, the presence of only two methyl groups on this nitrogen atom (as in N-methyladrenaline) is sufficient virtually to destroy activity
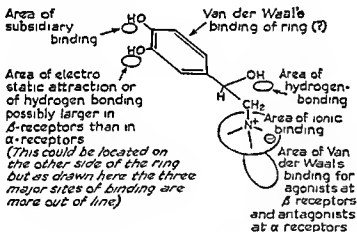
FIG 1X.9   Hypothetical structure of α- and β-adrenergic receptors

Any picture of the adrenergic receptors should indicate the points of attachment of the m hydroxyl group, the alcoholic hydroxyl, and the amino nitrogen atom, but even though the absolute configurations of (−)-noradrenaline and (−)-adrenaline are known, the molecule is so flexible that it is very difficult to do this with any confidence The situations at the α- and β receptors might be as depicted in Fig IX 9, but further information is clearly required and some of this might be obtained by a more detailed study of substances which block adrenergic receptors

ANTAGONISTS

Classification of Antagonists of Adrenaline

Some substances with affinity for adrenergic receptors but no (or little) efficacy have already been mentioned; the compounds with the large substituents on the nitrogen described on pages 296 and 304, which block the α-type of adrenergic receptor, and the 3 . 4-dichloro compounds discussed above, which block the β-type of receptor Many other compounds, less obviously related to adrenaline, also block α-receptors (but not β-receptors) and these can be classified first, according to their type of action, and secondly, according to their chemical structure
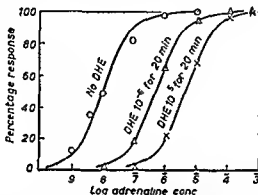
FIG IX.10 *Blockage of adrenergic receptors in the rabbit aortic strip by dihydro-ergotamine the graph of the logarithm of the concentration of adrenaline against the response is shown for different concentrations of dihydroergotamine The responses were measured when the tissue had been exposed to the blocking agent for 20 minutes (Furchgott, 1955, reproduced by permission)*

In the first group, the competitive antagonists, can be placed the ergot alkaloids, yohimbine, and certain imidazolines Furchgott (1954, 1955) found that in the presence of drugs of these groups, the dose-response curves for adrenaline (and/or *noradrenaline*) on rabbit aorta were parallel to the curve in the absence of antagonist, being displaced toward higher concentrations of agonist to an extent dependent on the concentration of the antagonist (Fig IX 10)

Drugs of the second group, notably the β haloalkylamines, also displace the dose-response curve for the agonist towards higher concentrations, but produce a block which is unsurmountable (Fig IX 11) These results are consistent with an irreversible blocking action of the type discussed on page 14, usually called 'non-competitive', but called by Furchgott an 'irreversible competitive' antagonism As Furchgott has pointed out, if only low concentrations of these antagonists are tested, the antagonism may be mistaken for competition If production of a maximum *response* by the tissue depended upon all the receptors being combined with agonist molecules, the irreversible combination of any receptors with antagonist molecules should render the tissue incapable of producing a maximal response however much agonist were given Such a situation is obtained, but only when many receptors have been blocked

irreversibly (Fig IX 11 indicates that a maximal response might still be obtained in the presence of low concentrations of antagonist) The results, accordingly, support the view that a maximal response of the tissues does not depend upon the combination of all the receptors with agonist molecules With low concentrations of antagonist there may still be sufficient 'spare' receptors for a maximum response to be obtained by high concentrations of agonist In these conditions the results closely resemble those obtained with a competitive antagonist
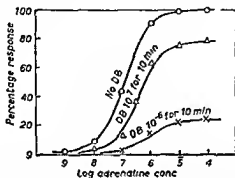
FIG IX 11 *Blockage of adrenergic receptors in the rabbit aortic strip by Diben-amine the graph of the logarithm of the concentration of adrenaline against the response is shown for different concentrations of Dibenamine The responses were measured when the tissue had been exposed to the blocking agent for 20 minutes (Furchgott, 1955, reproduced by permission)*

The actions of some competitive antagonists, on the other hand, may in these experiments be mistaken for non competitive antagonism, for the establishment of equilibrium conditions in these experiments may take a long time It appears as if there were a barrier between the drug in solution and the drug in the biophase, antagonism may take long to develop and equally long to pass off Furchgott (1955), for example, observed that with dibydroergotamine (see below) and the rabbit aortic strip, equilibrium took over an hour to be established, and when the preparation was washed its sensitivity was only half recovered at the end of 83 minutes

The action of some groups of compounds, benzodioxans, phenoxyethylamines, dibenzazepines, and hydrazinophthalizines (see below) have not been analysed in sufficient detail to allow their being definitely classified as competitive or non-competitive The work of Schild (1960), however, suggests that benzodioxans probably act competitively and, as phenoxyethylamines behave similarly in producing only relatively transient effects, it seems likely that these, too, are competitive antagonists

The Ergot Alkaloids

Ergot is a fungus which grows on rye It contains a variety of pharmacologically interesting substances iodiodiog choline, histamine, and a number of alkaloids The pharmacological effects of 'ergotoxine' were studied by Dale (1906), but this material has since been shown by Stoll and Hofmann (1943)

to consist of a mixture of three alkaloids, ergokrystine, ergokryptine, and ergocornine, of which the last named is the most important, comprising almost 90 per cent of the total Many other alkaloids are also present in ergot, and these can be divided into two series of isomers and each series can be divided into three groups (Table IX 14) The chemistry of the ergot alkaloids has been reviewed by Henry (1949), Glenn (1954), and Saxton (1960) and the pharmacology by Rothlin (1947)

TABLE IX 14
Ergot Alkaloids

First series	Second series	Broken down into		
Group I				
Ergotamine	Ergotaminine	(+)-Lysergic acid, NH₂, CH₃COCOOH, (+) proline	and	(−)-Phenylalanine (−)-Leucine
Ergosine	Ergosinine			
Group II				
Ergokrystine	Ergokrystinine	(+)-Lysergic acid, NH₂, Me₂CHCOCOOH, (+)-proline	and	(−)-Phenylalanine (−)-Leucine (+)-valine
Ergokryptine	Ergokryptinine			
Ergocornine (Ergotoxine)	Ergocorinine			
Group III				
Ergometrine (Ergobasine)	Ergometrinine	(+)-Lysergic acid	and	(+)-2 amino propanol

The amino-acids in the last column all belong to the 'natural' series and have the S-configuration, (+)-proline, however, has the R-configuration (but see page 323)

Stoll, Hofmann, and Becker (1943)

The members of the first series are laevorotatory and much more active than their isomers belonging to the second series, which are strongly dextrotatory and less soluble Although the compounds of both series can be broken down into (+)-lysergic acid, the members of the second series are considered to be derived from isolysergic acid, which can readily be converted into the tautomeric lysergic acid

Lysergic acid and isolysergic acid differ only in the arrangement of the carboxyl group and hydrogen atom at the 8 position In lysergic acid, the tetrahydropyridine ring is regarded by Cookson (1953) as being in a boat form with the carboxyl group axial but, according to Stenlake (1953, Stenlake and Raphael, 1953) a chair form is more likely to be correct (Fig IX 12) in either event, possibly both forms have comparable stability Hydrogenation of lysergic acid could yield two isomers, in one the hydrogen at the 10-position would be *cis* to the hydrogen at the 9 position and in the other it would be *trans* Because of the rigidity of the indole part of the molecule this hydrogen at the 5 position appears, inevitably, to be axial So far only one form of dihydrolysergic acid has been obtained, although two forms of dihydroiso-lysergic acid are known It seems likely (but not certain) that in dihydrolysergic

acid the hydrogen atom in the 10-position is axial and trans to the hydrogen atom in the 5-position
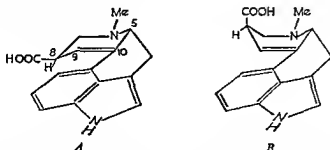
FIG IX 12   Stereochemistry of lysergic acid derivatives (+) lysergic acid A, after Cookson, B, after Stenlake

In isolysergic acid the H and COOH at the 8 position are interchanged

In dihydrolysergic acid or dihydroisolysergic acid the additional hydrogen atom at the 10-position can be either cis (a) to the hydrogen in the 5 position or trans (b)
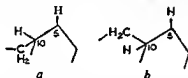
isoLysergic acid is much more readily converted into lysergic acid than is dihydroisolysergic acid into dihydrolysergic acid, and it is thought that the enol form (IX 55) of lysergic acid (or isolysergic acid) is stabilized by the conjugation of the double bond with the existing double bond between positions 9 and 10 In the dihydro derivatives there should be no such stabilizing influence, so interconversion of the tautomers should be more difficult

RCOOH = (+)-lysergic acid
R' = H,   R'' = PhCH₂-, (−)-Ergotamine, IX 56
R' = H,   R'' = Me₂HCCH₂-, (−)-Ergosine, IX 57
R = Me,   R'' = PhCH₂-, (−)-Ergokrystine, IX.58
R = Me,   R'' = Me₂HCCH₂-, (−)-Ergokryptine, IX 59
R = Me,   R'' = Me₂HC-, (−)-Ergocornine, IX 60

Enol form of lysergic acid, IX.55

Alkaloids of the 'inine' series are derived from (+)-isolysergic acid, e g RCOOH = (+)-isolysergic acid, R' = H, R'' = PhCH₂, (+)-Ergotaminine

Ergometrine and ergometrinine are much simpler in structure than the other alkaloids, being amides of (+) lysergic acid and (+)-isolysergic acid respectively with (+)-S-2 aminopropan-1-ol These compounds, however, have negligible blocking activity at adrenergic receptors, for which the more complicated polypeptide side-chain of the other groups of alkaloids is re-

quired The structures of these polypeptides have been worked out and structural formulae can, accordingly, be assigned to the alkaloids (IX 56–60) The (+) proline which is obtained by hydrolysis of the polypeptide (Table IX 14) appears to be due to inversion during hydrolysis, and it seems likely that the natural (−) S isomer is actually present in the alkaloid, for this is obtained when other methods of degradation are employed all the amino acids, in fact, appear to have the natural configuration The structure, however, is tricyclic, rather than bicyclic, for Stoll, Hofmann, and Petrzilka (1951) obtained a diketopiperazine derivative as a hydrolysis product in certain conditions The absolute configuration of part of the structure is therefore known, but the exact arrangement of the substituents of the oxazolidone ring is not

There are three main actions of the ergot alkaloids, they cause the plain muscle of the uterus to contract (an 'oxytocic' action), they cause an intense vasoconstriction, and they block the α type of adrenergic receptor Ergometrine possess the oxytocic properties without causing appreciable vasoconstriction or adrenergic block This suggests that the oxytic action is not connected with the vasoconstrictor or adrenergic blocking actions Ergometrine is particularly valuable in midwifery because it can be used to cause the uterus to contract without producing the other effects, which would be undesirable The vasoconstriction is very long lasting, and in people eating rye bread contaminated with ergot may lead to permanent arrest of the circulation ('St Anthony's Fire') and consequent gangrene, particularly in peripheral vessels, such as those of the toes This has long been regarded as being a 'direct' action of the alkaloids on the tissues, but Innes (1962) has suggested that it may be a stimulant action on the adrenergic receptors The compounds are considered to be partial agonists and the block of the adrenergic receptor to occur only when the receptors are saturated with these molecules with low efficacy

These results are inconsistent with those obtained by Furchgott (1955), but it must be pointed out that although graphs, such as those shown in Fig IX 10, appear to be strong evidence in support of an action by competition, they were not obtained in conditions of equilibrium but after exposure to the antagonist for only 20 minutes Ergot alkaloids do, in fact, have some stimulant *activity on the rabbit aortic strip, though the effects are much less than those* observed on blood vessels The mode of action of these compounds cannot, therefore, be regarded as being understood Indeed, because their actions take so long to reach a maximum effect and so long to pass off, it is not only virtually impossible to test whether they are competitive antagonists or partial agonists, but it is questionable whether these terms have any meaning The compounds are hardly ever used in conditions of equilibrium and the pharmacological activity is accordingly usually dependent upon the rate constants for uptake or, in some circumstances, for loss, and not upon the equilibrium constant

The slowness in the development of block is very difficult to reconcile with an action by competition The adrenergic receptor appears to be relatively *accessible to drugs because the response of the tissues to agonists is reasonably*

rapid a maximum effect is obtained with adrenaline on the rabbit aortic strip almost as rapidly as with acetylcholine and the frog rectus muscle The ergot alkaloids have a molecular weight about four times that of adrenaline, but lack the zwitterionic character of the latter and so might be expected to penetrate membranes, if anything, better than adrenaline If the alkaloids and adrenaline combine with the same receptors, why does the block take so long to develop?

More is known about the relationships between the chemical structure of the alkaloids and their oxytocic activity than about the relationships with blocking activity at the adrenergic receptors The oxytocic activity of ergometrine* appears to depend upon the presence of the (+) lysergic acid portion of the molecule The isomer derived from isolysergic acid, ergometrinine, is virtually inactive as are also the isomers derived from (—)-lysergic acid and (—)-R-2 aminopropan-1 ol, from (—)-lysergic acid and (+)-S-2 amino propan 1-ol, and from either (—)- or (+) isolysergic acid with either (—) or (+)- 2 amino-propan-1 ol The activity of the isomer derived from (+)-lysergic acid and (—) R 2 aminopropan 1-ol, however, was the same as that of ergometrine itself, but with the isomers of the synthetic compounds derived from (+)-lysergic acid and norephedrine, that derived from (—)-norephedrine was much more active than that derived from (—)-norephedrine (results of Rothlin, quoted by Stoll and Hofmann, 1953)

It would seem likely that blocking activity at the adrenergic receptors varies similarly with structure, activity being confined to derivatives of (+) lysergic acid with a polypeptide side-chain of one particular configuration The relative activities of ergot alkaloids and their dihydro derivatives in antagonizing the effects of adrenaline on the rabbit uterus and guinea pig seminal vesicles are shown in Table IX 15 The results indicate that activity is greatest with large substituents on the tricyclic polypeptide residue and that activity is considerably increased by hydrogenation As pointed out above, only one dihydro derivative is obtained on hydrogenation of the lysergic acid derivatives, in which the hydrogen atoms at positions 5 and 10 are probably *trans*, and there is no indication what the activity of the *cis* dihydro derivative is

Yohimbine Alkaloids and Related Compounds

Although yohimbine resembles derivatives of lysergic acid in that both might be derived from tryptophan, the formation of lysergic acid would involve cyclization with the 4-position of the indole ring, whereas the formation of yohimbine would involve cyclization with the 2 position Yohimbine is a derivative of tetrahydro*nor*harmane (IX 61) and closely related to the *Rauwolfia* alkaloids, such as reserpine Yohimbine has, in fact, been found to occur in various species of *Rauwolfia* (a shrub which grows in India), although

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* Note — The isomer of ergometrine derived from (+)-lysergic acid is only feebly laevorotatory, unlike the other ergot alkaloids of this series which are strongly laevo rotatory The isomer of ergometrine is, in fact, dextrorotatory in certain solvents it are certain salts including those listed in certain Pharmacopoeas In tests described in the British and United States Pharmacopoeas for example this isomer is dextrorotatory

TABLE IX 15

Antagonism of the Action of Adrenaline by Ergot Alkaloids

Alkaloids all derived from (+) form of lysergic acid	Equipotent molar ratios relative to ergotamine for antagonism of the actions of adrenaline on	
	Isolated guinea pig seminal vesicles	Isolated rabbit uterus
Ergotamine	1	1
Dihydroergotamine	0 14	0 44
Ergosine	1	1
Dihydroergosine	0 17	0 50
Ergokrystine	0 25	1
Dihydroergokrystine	0 029	0 29
Ergokryptine	0 25	0 67
Dihydroergokryptine	0 029	0 20
Ergocornine	0 5	2 0
Dihydroergocornine	0 04	0 4

Rothlin (1947)

the usual source is the bark of trees such as *Corynanthe Yohimbe* (which grows in Central Africa)

Yohimbine contains 5 asymmetric centres (at positions 3, 15, 16, 17, and 20), and it seems likely (review by Saxton, 1960) that formula IX 62 indicates
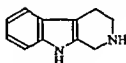
IX 61

the absolute configuration of (+) yohimbine The hydrogen atoms at the 3- and 15 positions and the hydroxyl group at the 17 position are axial on one side of the molecule and the hydrogen atoms at the 16 and 20-positions are axial on the other, the ester group being equatorial and the molecule as a
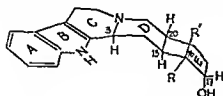
R = CO₂Me, R = H Yohimbine IX 62
R = H, R = CO₂Me, Corynanthine IX.63
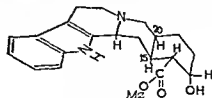
α Yohimbine IX 64

whole remarkably flat The alkaloid corynanthine (IX 63) is identical except that the ester group is axial instead of equatorial In β yohimbine the rings are again arranged in the same way, but both the ester and hydroxyl groups are equatorial

In α-yohimbine (IX 64) rings D and E are arranged differently, the hydrogen atom at the 20-position being *cis* to the hydrogen atom at the 15 position, the ester group is equatorial and the hydroxyl group axial (with ring E in the boat form) The same arrangement of rings D and E is found in reserpine, but the arrangement of rings C and D is also altered, the hydrogen atom at the 3 position being equatorial, both ester groups attached to ring E are equatorial and the methyl ether group is axial (IX 65) The alteration in the fusion of rings
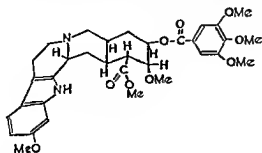
Reserpine, IX 65

C and D results in the folding of the molecule about the 3 4 bond The shape is quite different from that of yohimbine and it is not, therefore, surprising that in spite of the apparent chemical similarities between them, yohimbine and reserpine have quite different pharmacological properties

The blocking activity of yohimbine at adrenergic receptors is not very marked compared with that of the ergot alkaloids (Table IX 16) Yohimbine appears to be more active than corynanthine in antagonizing the effects of adrenaline on the blood-pressure (review by Bovet and Bovet Nitti, 1948), and hydrolysis of the ester group results in a considerable loss of activity A compound with an unsaturated ring E (IX 66), however, appears to be about one-

IX 66
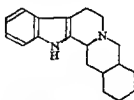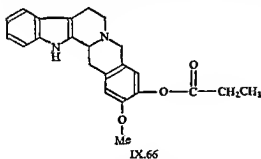
Desoxyyohimbol (rings
arranged as in yohim
bine), IX 67

quarter as active as yohimbine (Meli and Franchesini, 1957), and neither the ester group nor the alcoholic hydroxyl group of yohimbine appears to be essential, because appreciable activity is also shown by *desoxy* yohimbol (IX 67, Raymond-Hamet, 1943) Knbli, Balwani, Ray, and De (1957) found that α-yohimbine (rauwolscine) has blocking activity at the adrenergic receptors of the guinea pig seminal vesicles which is probably comparable with that

TABLE IX 16

Apparent* Association Constants for Drugs and Adrenergic Receptors in Rabbit Aortic Strip and Rabbit Ear

Antagonist	Time of exposure to antagonist (mins)	Agonist	Rabbit aortic strip	Rabbit ear
'Ergotoxine' (Ergocornine)	30	Adrenaline	—	2.5×10^5
Dihydroergotamine	30	Adrenaline or *nor*Adrenaline	5×10^7	—
	60	Adrenaline or *nor*Adrenaline	10^8	—
	240	Adrenaline or *nor*Adrenaline	2×10^8	—
Yohimbine	30	Adrenaline	—	3.3×10^5
	30	Adrenaline or *nor*Adrenaline	5×10^6	—
Tolazoline	30	Adrenaline	—	3.3×10^5
Phentolamine	30	Adrenaline	—	5×10^7
	20	Adrenaline	3.3×10^7	

* These constants are called 'apparent' because they were not determined in equilibrium conditions

Results with the rabbit ear were obtained by *Fleckenstein (1952)* and with the rabbit aortic strip by *Furchgott (1955)*

of yohimbine, although the results of Hesse and Lauger (1931) indicated that it is less active than yohimbine in lowering the blood pressure of the rabbit

The properties of reserpine are quite different It has no significant ability to block adrenergic receptors (review by Bein, 1956) The fall in blood-pressure which it produces takes time to develop and appears to be brought about chiefly by an action on the mechanism by which catecholamines (and other amines, such as 5-hydroxytryptamine) are stored, though the compound also has effects on the central nervous system

Phenoxyethylamines and Benzodioxans

The oxytocic action of the compound *Gravitol* (IX 68) was described by Eichholtz (1928) and by Schmidt and Scholl (1928), but the work of Anan and Okazaki (for references, see Fourneau and Bovet, 1933) indicated that this compound, and compounds related to it, also antagonized some of the effects of adrenaline Phenoxyethyldiethylamine itself (*F 928*, IX 69) could be used to block the pressor actions of adrenaline, revealing the fall in pressure produced by the action of adrenaline on β-adrenergic receptors (Levy and Ditz,

1934) This compound and the thymyl ether (*F 929*, IX 70) also antagonized the effects of histamine (Bovet and Staub, 1937, Staub, 1939) and are interesting because it is from them that the first useful antihistamine drugs were de-
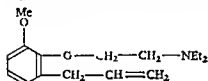
Gravitol, IX 68
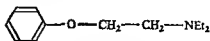
F 928, IX 69

veloped Fourneau and Bovet (1933) observed that basically substituted benzodioxans also antagonized the effects of adrenaline, the compounds *F 883* (*Prosympal*, IX 71) and *F 933* (*Piperoxan*, IX 72) being the most active of the compounds studied and more active than *F 928* (Bovet and Maderni, 1933, Bovet and Simon, 1937) Trefouel, Trefouel, and Dunant (1935) have re-
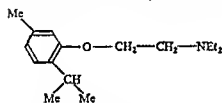
F 929, IX 70
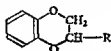
R=CH·NEt₂ , *F883* , IX 71,

R=CH₂N◯ , *F933* , IX 72

solved *F 883* and the (−) isomer has been found to be more active than the (+)-, comparable effects in blocking the pressor actions of adrenaline on the cat blood pressure were obtained with about six times as much of the latter as of the former (Bovet and Simon, 1935)

The activity of *F 883* and *F 933* in blocking the α adrenergic receptors is low compared with that of the ergot alkaloids, and of much shorter duration The compound *Dibozane* (IX 73), however, is appreciably more active, particularly
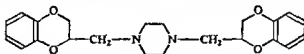
Dibozane, IX 73

when tested for ability to suppress the carotid occlusion reflex rather than for ability to antagonize the effects of adrenaline (O'Leary, 1953) Its effects in man are rather variable (Rosenblatt *et al* , 1954), but the compound has been used by Ahlquist and Levy (1959) and by Levy (1959) in order to block α adrenergic receptors in experiments on the blood pressure and intestine of the dog Rapela and Green (1961) have examined its effects in blocking adrenergic receptors in the skeletal muscles of the dog and compared them with those produced by *Phenoxybenzamine* and *Azapetine* (see below) *Dibozane* and *Phenoxybenzamine* produced effects in comparable doses which were about one third, or less, of the dose of *Azapetine* required The blockade produced by *Dibozane* and *Azapetine*, however, lasted for a shorter time than that produced by *Phenoxybenzamine*, and with all three drugs the block was much

more difficult to surmount with higher doses of antagonist This increase in the drug ratio (page 43) with the amount of antagonist suggests that the antagonism is non competitive rather than competitive

Imidazolines

The effects of 2 substituted imidazolines on blood vessels were studied by Hartmann and Isler (1939) It was found that 2 alkylimidazolines, in which the alkyl group was from 6 to 8 atoms in length (IX 74), and 2 phenyl-alkylimidazolines, such as *Tolazoline* (*Priscol*, IX 75), caused vasodilatation and a fall in blood pressure The exchange of phenyl for α naphthyl, as in *Naphazoline* (*Privine*, IX 76), or β indolyl (IX 77) or the introduction of phenolic or methoxy groups in the benzene or naphthalene ring, altered the activity from depressor to pressor The most potent of these latter compounds, *Phedracin* (IX 78) appeared to have effects on the blood pressure intermediate between *Neosynephrine* (page 302) and ephedrine in both duration and intensity (Elmes and Jefferson, 1942)

R = C_6H_{13}—C_8H_{17}, IX 74,

R = $PhCH_2$, *Tolazoline*, IX 75

R = [naphthyl], *Naphazoline*, IX 76,

R = [indolyl], IX 77,

R = [MeO-substituted benzyl], *Phedracin* IX 78
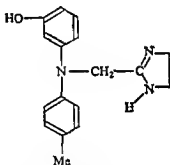
Phentolamine IX 79

The vasodilatation produced by *Tolazoline* is accompanied by, and may partly be due to a block of α adrenergic receptors Blocking activity is relatively feeble (Table IX 16) but the block appears to be competitive (Nickerson, 1949, Furchgott, 1955) The related compound *Phentolamine* (*Regitine*, IX 79) is more active Roberts Richardson, and Green (1952) found that comparable degrees of block of the α adrenergic receptors of the dog's hind limb were produced by doses of *Phentolamine* which were only one fifth to one seventh of those of *Tolazoline*, but on the rabbit aortic strip and perfused rabbit ear the equipotent molar ratio for *Tolazoline* relative to *Phentolamine* appears to be about 100 (Table IX 16) Like *Tolazoline*, however, *Phentolamine* does not act only at α adrenergic receptors, it also has some activity at cholinergic receptors and directly on the tissues themselves

Dibenzazepines

The compound *Azapetine* (*Ilidar*, IX 80) was the most active adrenergic blocking agent of a series of dibenzazepines studied by Randall and Smith (1951)

a particularly stable complex with the receptors It is not strictly accurate to describe the process as 'irreversible' but, as described on page 13, the block appears to be consistent with a non-competitive antagonism (Nickerson, 1949, 1957, page 371) It seems reasonable to suppose that the iminium or vinyl ammonium compounds alkylate a group on the receptor and that this covalently linked product is relatively stable at body temperature Harvey and Nickerson (1954) have studied the actions of compounds related to *Dibenamine* on substances containing sulphydryl, amino, carboxyl, and phosphate groups and concluded that the action at the receptor was most likely to involve alkylation of a sulphydryl group Direct evidence of this might conceivably be obtained by experiments similar to those performed with *DFP* and cholinesterases (page 274), but the impossibility of purifying and concentrating adrenergic receptors, as one can cholinesterases, would make this a formidable task

In view of the ability of the compounds to act as alkylating agents, it is not surprising that they have been found to combine with a wide variety of tissues besides the adrenergic receptors They have been shown to block cholinergic receptors and histamine receptors (see, for example, Nickerson, 1949, Graham and Lewis, 1953, Furchgott, 1955) and their ability to react with tissues generally, rather like the 'nitrogen mustards', limits their therapeutic value What is, perhaps, remarkable is that their effects on the blood pressure are primarily dependent only upon their actions at a adrenergic receptors

It is difficult to obtain quantitative estimates of the relative adrenergic blocking activity of compounds acting like *Dibenamine* Nickerson and Gump (1949) and Nickerson and Nomaguchi (1951) used only a qualitative assessment of activity on the blood pressure, the compounds were classified into those which were distinctly more active than, roughly equal to, and distinctly less active than, *Dibenamine* Loew and Micetich (1948) compared estimates of the doses of the compounds which required 50 per cent of a group of mice from the effects of a large dose of adrenaline Fellows *et al* (1954) compared estimates of the 'paralysing doses' of the compounds, these being the amounts which abolished the effects of a standard dose of adrenaline on the blood pressure Graham (1947) has made similar comparisons using the 'ED_{50}', this being the dose which antagonizes the effects of a standard dose of adrenaline on the blood-pressure of 50 per cent of a group of animals The significance of these quantitative estimates may not be very great Fellows *et al* (1954) recorded variations of 2 fold or more in the paralysing dose, but the results should serve to indicate the order of activity of the compounds

Many compounds are considerably more active than *Dibenamine*, *Phenoxybenzamine* (*Dibenzyline*, IX 82), in particular, is highly effective, both orally and intravenously, in doses of about one tenth of those of *Dibenamine* (Nickerson and Nomaguchi, 1951, Fellows *et al*, 1954) The high potency of phenoxyethyl derivatives is of particular interest in view of the blocking activity of the compounds discussed on page 328, but there may be no connexion between the actions of the two groups of drugs, because there is evidence (Belleau, 1958, 1959) that in the former the ether oxygen atom interacts with a carbon atom in the ethylene iminium ring

The effects on activity of substituents on the benzene ring have been reviewed by Belleau (1958), who has suggested that groups which produce +E or +I effects will favour activity, provided they do not interfere with binding at the *p*- and *m*-positions of the ring. The electron distribution in these molecules should be similar to that in adrenaline, in which the *m*- and *p*-hydroxyl groups should increase the availability of electrons in the benzene ring.
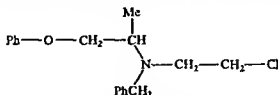
Phenoxybenzamine, IX 82

Groups which lead to a withdrawal of electrons from the ring should decrease activity. This idea is essentially the same as that of Nickerson and Gump (1949), for an electron-withdrawing group should decrease the stability of the ethylene iminium ion, whereas electron releasing groups should increase its stability. Belleau (1959, 1960) has also considered the steric factors involved in the fit of the ion to the receptor and supposes that the ethylene iminium can be considered as analogous to the adrenaline ion (Fig IX 14). After the initial
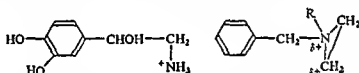
FIG IX 14 *Comparison of the adrenaline ion with an N benzylethylene iminium ion*

adsorption at the receptor, alkylation should follow very rapidly, and Belleau has suggested that this may involve a carbonate or phosphate group.

Chapman (1960) and Graham and James (1961) have described some compounds which form ethylene iminium ions in which the aromatic group is attached to a carbon atom instead of to nitrogen. Some of these, e g 2-bromo-2 (*p*-bromphenyl)-ethyldimethylamine (IX 83, Table IX 17), are very
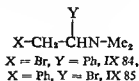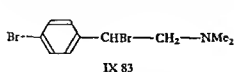
highly active, producing effects in doses about one-teothousandth of those of *Dibenamine* and having an affinity for the receptors several times that of adrenaline itself. These results indicate that 0 6 nanomoles of the compound can block the action of 5 nanomoles of adrenaline. It is interesting that 2-bromo-1-phenyl-ethyldimethylamine (IX 84) has about the same activity as the isomeric 1 bromo-2-phenyl-ethyldimethylamine (IX 85), this would be expected because both should form the same ethylene iminium ion. Chapman

TABLE IX.17

Adrenergic Blocking Properties of 2 phenyl 2 halo-ethylamines

$$\overset{R}{\underset{R}{>}} N\cdot CH_2 CH \underset{X}{\overset{}{\bigcirc}} Y$$
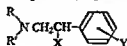
R	R	X	Y	ED_{50} (μmoles/kg) on rat blood pressure for antagonism of	
				(−)-Adrenaline (5 nmoles/kg)*	(−)-norAdrenaline (3 nmoles/kg)*
Me	Me	Cl	H	0 046	0 1
Me	Me	Br	H	0 035	0 056
Me	Me	I	H	0 022	0 015
Et	Et	Br	H	5	1 8
n Pr	n Pr	Br	H	1	t 3
IsoPr	IsoPr	Br	H	30	22
allyl	allyl	Br	H	0 03	0 03
Me	H	Br	H	0 6	0 5
Et	H	Br	H	100	80
n Pr	H	Br	H	250	250
Pipendino		Br	H	15	14
Pyrrolidino		Br	H	0 05	0 08
Me	Me	Br	p-Br	0·0006	0 0002
Me	Me	Br	p-Me	0 0003	0 0007
Dibenamine				7 7	10
Phenoxybenzamine				0 28	0 55
Me₂NCHPh CH₂Br (IX 84)				0 04	0 05

* 1 nmole = 10⁻³ μmole

ED_{50}. The dose preventing the response to adrenaline in 50 per cent of the animals (tested when the block appeared to be at its peak) The antagonism of adrenaline and noradrenaline should be the same if the experiments are assessing only activity at α adrenergic receptors Although the doses of adrenaline and noradrenaline are different the responses should be similar and anyway the block appears to be non-competitive

Graham and James (1961)

(1960) has suggested that with these compounds, as with compounds related to *Dibenamine* it is the carbonium part of the ethylene iminium ion which be comes attached to the receptor, rather than the nitrogen atom (Fig IX 15) It might be supposed that substitution of a bromine atom in the *p* position would increase the stability of the carbonium ion, whereas substitution of a methoxyl group in this position would decrease the stability The pharmaco logical activities of these compounds however, are not very different which suggests that steric factors must also be considered

In contrast to compounds related to *Dibenamine*, these compounds pro duce effects which are rapid in onset and of relatively short duration Graham

and James (1961) considered that the antagonism was probably non competitive because it was unsurmountable, i e could not be reversed by increasing the dose of adrenaline or noradrenaline The shortness of the block, compared with that produced by *Dibenamine*, could well be due to the difference in the
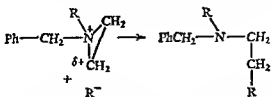
FIG IX 15   *Attack on an α adrenergic receptor by an ethylene iminium ion*

stabilities of the products formed by alkylation of the receptor With *Dibenamine* the receptor should become combined with a dialkylaminoethyl group, whereas with the latter compounds it is probably combined with a 2 dimethyl amino 1 aryl group (Fig IX 16), which might be expected to be broken down much more easily
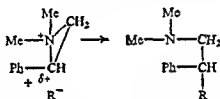
FIG IX 16   *Attack on an α adrenergic receptor by an ethylene iminium ion formed from 2 bromo 2 phenylethyldimethylamine*

Nature and Function of Adrenergic Receptors

The consideration of the relationships between chemical structure and ability to block adrenergic receptors does not yield as much information about the nature or function of the receptors as might have been hoped Nothing further can be added to what has already been deduced about β receptors (page 318)

The blocking activity of *Phentolamine* at α receptors is consistent with the picture shown in Fig IX 9, but the compounds with the greatest blocking activity at α receptors are the ergot alkaloids especially dihydroergokrystine and dihydroergokryptine, and derivatives of 2 bromo 2 phenyl ethyldimethylamine How the ergot alkaloids become attached at the α adrenergic receptors is rather obscure The only basic group in the molecule is that of the tetrahydropyridine or piperidine nitrogen atom in the lysergic acid or dihydrolysergic acid part of the molecule The pK_a of this is different in the various derivatives, but is usually about 7 (6 8 for ergometrine, 7 3 for ergometrinine, 7 8 for lysergic acid, and 8 4 for isolysergic acid at 24°, Craig Shedlovsky, Gould, and Jacobs, 1938) Possibly the benzene ring of the mole cule is adsorbed at the receptor with this basic group (as a cation) in place of the ammonium group of adrenaline and the $-NH-$ group of the indole ring in place of the *m* phenolic group, but the major attachment of the molecule

seems likely to involve the polypeptide side-chain and consequently to occur at points on the receptor surface remote from the receptor groups which form the points of attachment of adrenaline and *noradrenaline*

In the alkylation of α-adrenergic receptors by compounds such as the N-benzyl-N-phenoxyethyl-ethylene iminium ion (formed from *Phenoxy benz amine*) or the N N-dimethyl-2-phenyl-ethylene iminium ion (formed from 2-bromo-2-phenylethyldimethylamine), the attack, as already mentioned, appears to involve the carbonium ion rather than the nitrogen atom Belleau (1958, 1959) has emphasized the importance of the distance between this carbonium ion and the benzene ring and the value of substituents in the *m-* or *p-*positions His conclusions about the relationships between chemical structure and blocking activity of this type are again consistent with the picture of the receptor in Fig IX 9, but do not yield any further information about the nature of the receptor groups or their relative positions It should, in fact, be pointed out that the results of Harvey and Nickerson (1954) led them to conclude that alkylation involved a sulphydryl group, whereas it is usually assumed that the ammonium group of adrenaline or *noradrenaline* is bound to a carboxyl or phosphate group (or some other anionic group)

The action of adrenaline and *noradrenaline* in increasing oxidative phosphorylation by catalysing the breakdown of adenosine triphosphate to cyclic adenosine monophosphate (page 283) has been supposed by Belleau (1960) to involve the formation of a complex formed by an adrenaline molecule, an adenosine triphosphate molecule, and a divalent metal ion The divalent metal ion is considered to form a chelate complex involving the two phenolic groups of adrenaline and the second and third phosphate groups of adenosine triphosphate, the cationic ammonium group in adrenaline being attracted to the first phosphate group Although the oxidation of adrenaline to adrenochrome is catalysed by metal ions (adrenaline solutions containing ethylene diamine tetraacetic acid can be kept for long periods), the complex with adenosine triphosphate, as postulated by Belleau, does not involve the hydroxyl group at all. The reaction, nevertheless, appears to be stereospecific, about 20 molecules of (+) adrenaline being needed to produce the effects of 1 molecule of (−)-adrenaline (Sutherland and Rall, 1960), so unless the hydroxyl group is involved in the link to the protein, this picture may have to be modified Its bearing on the physiological actions of adrenaline, other than in stimulating glycolysis, is questionable, because, as has already been pointed out (page 288), although increased breakdown of adenosine triphosphate might conceivably stabilize the smooth muscle membrane and depress excitability, it is difficult to see why exactly the same process should increase the excitability of other tissues, such as heart muscle Although the occurrence of catecholamines and adenosine triphosphate together in the granules (page 283) suggests that the actions of the former may involve the latter, it cannot be regarded as evidence that this is necessarily so The basic substance histamine is stored in Mast cells along with the acidic substance heparin (see page 345), but there is no suggestion that the actions of these compounds are linked together

Effects Produced by Blocking the Destruction of Adrenaline and Noradrenaline:

As has already been mentioned (page 285), the inactivation of the sympathetic transmitter is more complicated than that of acetylcholine It appears to involve mostly O-methyl transferases, but inactivation by oxidation may also occur to a smaller extent Work on the isolation and properties of catechol-O-methyl transferases is still only in its preliminary stages It appears that these enzymes are inhibited by pyrogallol and, as long ago as 1936, Bacq observed that this substance potentiated the effects of adrenaline both in causing contracture of the cat's nictitating membrane and in producing inhibitory responses, such as relaxation of the cat's uterus The doses were very high, however, and so far no compounds have been produced which can be regarded as imitating the actions of adrenaline and *noradrenaline* by blocking their destruction by O-methyl transferases.

There is more information about amine oxidases (reviews by Blaschko, 1952, Davison, 1958, Zeller, 1951, 1960,) but even these have not been studied as extensively as the cholinesterases At least two families of enzymes can be distinguished, monoamine oxidases and diamine oxidases The former only oxidize polymethylenediamines (IX 86) in which there is a considerable distance between the two amino groups, as in the deca-
methylene to hexadecamethylene compounds, $\quad H_2N-(CH_2)_n-NH_2$
diamine oxidases only oxidize these long chain com- $\qquad$ IX 86,
pounds very slowly and are most active in oxidizing $\quad n = 4$, Putrescine, IX 87,
those with a short distance between the amino $\quad n = 5$, Cadaverine, IX 88
groups, as in putrescine and cadaverine (IX 87 and
88, Blaschko and Hawkins, 1950) Most monoamine oxidases appear to be insoluble, being bound to the mitochondria of the cell wall They do not appear to contain prosthetic groups or to require coenzymes A soluble amine oxidase obtained from pea-seedlings has been obtained in a highly purified state by Mann (1961), but this resembles a diamine oxidase rather than monoamine oxidase in that it is inhibited by semicarbazide or cyanide ions, and also by chelating agents (Hill and Mann, 1962) A naturally occurring soluble form of monoamine oxidase has also been described by Weissbach, Redfield, and Udenfriend (1957), and though it appears, from its substrate specificity to be a true monoamine oxidase, in the guinea-pig liver it is neither as plentiful nor as easily obtained as the insoluble enzyme associated with it The 'insoluble' enzyme can, however, be freed from the mitochondria and obtained in solution Blaschko (1952) has described the use of the enzyme lysolecithinase to digest the mitochondria and leave the amine oxidase in 'solution', but it appears that this is not a true solution and that the enzyme is still associated with insoluble material, though the size of the particles is very small Cotzias, Serlin, and Greenough (1954) have described a method of separating the enzyme from the mitochondria by the addition of a suitable detergent and Zeller *et al* (1958) have described a method in which the separation is achieved by ultra-sonic vibration All these procedures, however, are much more complicated than the use of suspensions of washed mitochondria
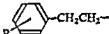
and most of the studies of the substrate specificity of the enzymes and the effects of inhibitors have been performed with crude preparations of this type In these circumstances, the results may be complicated by the uptake of oxygen by the products of the oxidation of the amine and it may be necessary to ignore results which have been obtained over a long period of time and to add substances, such as cyanide ions, which stop further oxidation of the compounds beyond the aldehyde stage

Unlike the adrenergic receptors, amine oxidases are not particularly stereospecific Blaschko, Richter, and Schlossman (1937) observed that enzyme preparations from guinea-pig liver oxidized (—)-adrenaline at about twice the rate of (+)-adrenaline and similar results indicating only moderate stereospecificity have been obtained by Pratesi and Blaschko (1959) The experiments also revealed differences between the activities of enzyme preparations made from different sources With rabbit liver preparations R- and S-β hydroxyphenylethylamine (IX 33) were oxidized at the same rate and (±)- and *S-S)ympatol* (IX 22) were both oxidized at about half this rate With preparations from guinea pig liver (±)- and *S-Sympatol* and (—)- and (±)- *Metasympatol* (IX 14) were all oxidized at about the same rate, the R-isomer of β-hydroxyphenylethylamine more slowly, and the S-isomer more slowly still

Some idea of the substrate specificity of amineoxidases can be obtained from Table IX 18 There is a considerable difference between the results obtained

TABLE IX 18
Substrate Specificity of Amineoxidases of Guinea pig and Cat Liver

R—⟨benzene⟩—CH₂CH₂—	Rate of oxidation relative to that of tyramine during the first 20 minutes of reaction					
	NH₂		NHMe		NMe₂	
	Guinea pig	Cat	Guinea pig	Cat	Guinea pig	Cat
4-OH	100	100	92	98	5	40
3-OH	69	70	121	83	18	50
2 OH	16	64	38	63	2	30
3 4-(OH)₂	122	91	113	82	19	29
Adrenaline	—	—	40	18	—	—
H	19	25	63	85	5	16
4-OMe	7	39	23	83	11	24
3-OMe	31	21	65	62	11	16
2-OMe	94	79	93	68	12	45
3 4-(OMe)₂	10	25	12	43	8	9

The substrate concentration was 12 mM in all experiments and cyanide ions were present to inhibit oxidation of the amine beyond the aldehyde

Randall (1946)

tained with guinea-pig liver and with cat liver, but there is agreement enough to indicate that the active centres of the enzymes are much less specific than the adrenergic receptors in the tissues The oxidation appears to depend upon two features, the amino group and the 3- or 4-phenolic group With the enzyme from rabbit liver the results of Alles and Heegaard (1943) and of Pratesi and Blaschko (1959) indicate that even the latter is unnecessary, β phenylethylamine being oxidized more rapidly than tyramine It must be remembered, however, that changes in the rate of oxidation can be brought about by changes in the rate constant for the breakdown of the enzyme-substrate complex, as well as by changes in the Michaelis–Menten constant Blaschko and Pratesi found that the enzymes of guinea-pig liver actually had a higher affinity for β-phenylethylamine than for tyramine, even though the latter has oxidized more rapidly The presence of a β-hydroxyl group, as in adrenaline, appears to lower the rate of oxidation These results are all consistent with the low degree of stereospecificity which has already been commented on

The introduction of methyl group α- to the amino group makes the compounds resistant to the actions of amine oxidases, but they retain affinity for the enzymes and act as inhibitors The inhibitory effects of ephedrine were observed by Blaschko, Richter, and Schlossman (1937), and Mann and Quastel (1940) have shown that the inhibition appears to be a reversible competition For amine oxidase of guinea-pig liver the Michaelis–Menten constant of tyramine appears to be 1.8×10^{-3} M (Blaschko, Richter, and Schlossman, 1937, results obtained by Barlow, 1961, give a value of approximately 1.2×10^{-3} M) and the inhibitor constant for (−)-ephedrine is 5.7×10^{-3} M The (−) isomer of ephedrine is a slightly more potent inhibitor than the (+) isomer, but there is not much difference, and (+)-ψ-ephedrine appears to have comparable activity (Blaschko, 1938) Compounds without the β-alcoholic group are more potent, Mann and Quastel (1940) found the inhibitor constant for Amphetamine and the enzyme from guinea-pig liver to be 4×10^{-4} M With preparations from rabbit liver, the inhibitory activity of the two isomers of Amphetamine is the same (Pratesi and Blaschko, 1959), but with preparations from guinea pig liver the (+) isomer is slightly more active than the (−)-isomer The substance 1-phenylethylamine (IX 89) appears to have activity comparable with that of Amphet-amine (Grana and Lilla, 1959) The resistance of these α-methyl compounds to oxidation, although they have a high affinity for the enzyme, has been taken to indicate that one hydrogen attached to the α carbon atom of the substrate must come into close contact with the enzyme and that a second hydrogen atom is involved in the dehydrogenation the presence of a methyl group would prevent this (Zeller, 1960) The presence of a methyl group on the β carbon atom does not confer resistance to oxidation (Beyer and Morrison, 1945)

$$\begin{array}{c} NH_2 \\ | \\ Ph\text{-}CH\text{-}CH_3 \\ \text{IX 89} \end{array}$$

More potent inhibitors of amine oxidase have been developed Zeller *et al* (1952) found that N-isopropyl-N-isonicotinylhydrazine (*Iproniazid, Marsilid,* IX 90) produced 77 per cent inhibition of the oxidation of tyramine

$(5 \times 10^{-2} \text{M})$ by mitochondria from rat liver in a concentration as low as 4×10^{-5} M The inhibition, however, is not competitive (Zeller, Barsky, and Berman, 1955, Davison, 1957) The inhibitor appears to react with the en-
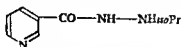
Iproniazid, IX 90

zyme to form a stable complex which is not broken up by dialysis This reaction takes time (around 6 to 12 minutes), but must occur at the active centre because the presence of tyramine prevents the action of the inhibitor The process can be regarded as non competitive and the pI_{50} for rat liver mitochondria is 4 9 Many hydrazides have similar properties and are more active The pI_{50} for isopropyl hydrazine (IX 91) is 5 8, and for 2 phenylisopropyl hydrazine (*Pheniprazine, Catron*, IX 91, Horita, 1958) the value is greater than 6
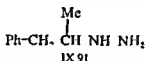
IX 91

The most active compounds yet known are the *cis* and *trans* forms of 2 phenyl*cyclo*propylamine (IX 92 and 93) Sarkar *et al* (1960) using beef liver mitochondria obtained estimates of the pI_{50} of around 7 2 for the *trans* compound (*Tranylcypromine*) and slightly less for the *cis* compound, whereas for 1-phenyl*cyclo*propylamine the values were 5 1 and *cyclo*propylamine itself was only feebly active (Zeller, 1960)
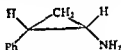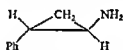
IX.92   *Tranylcypromine*, IX 93

Although these *cyclo*propyl derivatives and hydrazides are very much more active than ephedrine and Amphetamine as inhibitors of amine oxidases, they do not produce the same sympathomimetic effects as these compounds and it is now, therefore, quite clear that ephedrine and Amphetamine do not act by preventing the destruction of adrenaline and/or noradrenaline

Drugs which Act at Sympathetic Nerve Endings

The action of cocaine in potentiating the effects of some compounds, such as adrenaline, and decreasing the effects of others, such as tyramine (Table IX 9), has long been a mystery Burn and Robinson (1952) suggested that this might be due to an inhibition of amine oxidase Although Philpot (1940) demonstrated that cocaine inhibited the *in vitro* oxidation of tyramine by preparations of rat and guinea pig liver, high concentrations $(10^{-1}$ to 10^{-2} M) were needed, and it is now clear that some other explanation is necessary because destruction by amine oxidase is not primarily responsible for terminating the

actions of adrenaline Axelrod (1960), for example, has found that cocaine had no effect on the rates of disappearance of isotopically labelled adrenaline and noradrenaline from mice

The evidence that *noradrenaline* is stored at the nerve-endings indicates another site where substances could act Macmillan (1959) has suggested that cocaine acts on these stores, blocking the release of *noradrenaline* from them and the uptake of *noradrenaline* into them Blockage of the uptake of *noradrenaline* would potentiate the effects of *noradrenaline* because it is considered that the rapid return of *noradrenaline* to the store is normally responsible to a considerable extent for limiting its actions The actions of tyramine are presumably antagonized because these depend upon the release of *noradrenaline* from the stores It certainly appears that compounds like tyramine, which do not produce sympathomimetic effects in animals treated with reserpine, will produce effects after an infusion of *noradrenaline* and that these effects are antagonized by cocaine either in the normal animal or in that treated with reserpine followed by an infusion of *noradrenaline* (Farrant, 1960) This explanation, however, suggests that the actions of all substances which are potentiated by cocaine are limited by uptake at sites of storage The experiments of Burn and Rand (1960) indicate that the stores at the nerve-endings do not contain adrenaline, but it is possible that compounds other than *noradrenaline* are taken up at other 'sites of loss' (Vane, 1961) and that this process is also blocked by cocaine

Recently compounds have been discovered which interfere with the release of the sympathetic transmitter (reviews by Bain, 1960, and Bein, 1960) The first of these, choline-2 6-xylyl ether (IX 94), was an addition to the group of
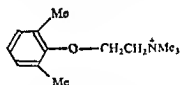
IX 94   Bretylium, IX 95
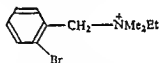
phenyl ethers of choline originally studied by Hey (1952) and discussed in *Chapters V and VI It was found to produce a prolonged fall in blood-pressure*, but was shown apparently to have local anaesthetic properties (Hey and Willey, 1953, 1954) Exley (1957) found that, in fact, the compound does not block conduction in nerve trunks and has no action on parasympathetic connexions (apart from a transitory blockade of ganglia) Its actions appeared, therefore, to be exclusively at the sympathetic nerve endings where it interfered with the release of transmitter, this can easily be shown with the Finkleman preparation (page 292) in which the response of the gut to sympathetic stimulation is blocked although the responses to adrenaline are unaffected Other compounds with this type of action have been described (Boura, Copp, and Green, 1959, Boura *et al*, 1960) of which *Bretylium* (IX 95) and *BW 172 C 58* (IX 96) are particularly active Studies with isotopically labelled *Bretylium* show that this compound is actually selectively

taken up by sympathetic ganglia and postganglionic sympathetic nerve-trunks

Somewhat similar results have been obtained with the substance *Guanethidine* (IX 97, Maxwell, Mill, and Plummer, 1959, Maxwell *et al*, 1960) which
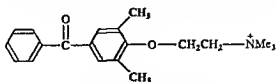
BW 172 C 58, IX.96

was developed from the less active *Su-4029* (IX 98, Maxwell, Ross, and Plummer, 1958) The effects are those of sympathetic blockade although the receptors are still sensitive to *noradrenaline* and adrenaline These compounds, therefore, like *Bretylium*, can be regarded as producing a pharmacological sympathectomy and, as is observed with sympathectomy, the sensitivity to added *noradrenaline* and adrenaline tends to increase when the block has been established for some time
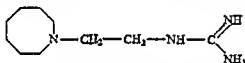
Guanethidine, IX.97

These compounds have been used clinically for lowering the blood pressure in patients As they prevent the release of the transmitter they might be very effective when the cause of the raised blood pressure is a high sympathetic tone *Guanethidine*, for instance, is very effective in lowering the blood pressure of dogs in which it has been raised by interference with the circulation to the kidney, whereas it has no effect on normal animals The exact therapeutic value of these compounds has yet to be assessed, but results with *Bretylium* have not been as satisfactory as might have been expected
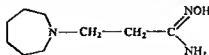
Su-4029, IX 98

How these compounds act at the nerve-endings is not clear Burn (1961) has suggested that the release of transmitter actually involved a cholinergic mechanism, that acetylcholine is released first and that this causes the release of *noradrenaline* from the stores Substances like *Bretylium* are considered to prevent this action of acetylcholine There is no direct evidence for this Thompson (1960) and Gardiner and Thompson (1961) have failed to find evidence in sympathetic nerve-endings of acetylcholine or acetylcholinesterases, but this cannot be taken as positive evidence against the hypothesis, even though it suggests that it is not very likely

In addition to actions on the adrenergic receptors and on storage mechanisms, compounds may effect blood-pressure by actions at sites such as the sympathetic ganglia (already discussed), at sensory nerve-endings (producing reflex effects), in the central nervous system or directly on the vessels themselves Some compounds which have been used in the treatment of high blood-pressure, in fact, appear to act directly on the blood-vessels and/or on the central nervous system Hydrazinophthalazine (*Hydrallazine*, *Apresoline*, IX 99, and Gross, Druey, and Meier, 1950, Freis and Finnerty, 1950) and 1 4-dihydrazinophthalazine (IX 100), for example, produce a long lasting

Hydrallazine, IX 99

IX 100
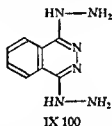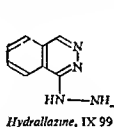
fall in blood-pressure by an action which may partly be central (Moyer, Huggins, and Handley, 1953), but is predominantly peripheral (Ablad, 1959) Hydrazinophthalazine has virtually no blocking activity at adrenergic receptors (Tripod and Meier, 1954), and although a powerful inhibitor of amine oxidases, this action appears to be unrelated to its effects on blood-pressure (Gross, Sebuler, Tripod, and Meier, 1952, Sebuler and Wyss, 1960)

The action of reserpine is also extremely complicated, involving central effects as well as actions on the storage of catecholamines and 5-hydroxytryptamine (see, for instance, Robson and Stacey, 1962), it has, however, little blocking activity at adrenergic receptors (Tripod and Meier, 1954)

Conclusion

It is possible to distinguish between two main types of adrenergic receptors and from the relationships between chemical structure and pharmacological activity to conjecture how the binding groups may be arranged within the receptor These pictures of the receptors, however, are even more hypothetical than those of acetylcholine receptors This is partly because there is much less satisfactory information from antagonists at adrenergic receptors than at acetylcholine receptors There is (as yet) no class of compounds which potentiates the actions of the sympathetic transmitter by preventing their destruction, but there are compounds which appear to mimic the actions of *noradrenaline* by causing its release from stores There are also substances which block the release of sympathetic transmitters and others which appear to prevent their uptake by the stores

M

X

Direct Actions on Tissues:
Drugs affecting Histamine Receptors

Introduction

The sites so far considered have all been part of the peripheral nervous connexions of the body   From the therapeutic point of view, drugs acting at these sites are relatively unimportant compared with those affecting the central nervous system or acting directly on various organs in the body, or on parasites in the body which produce disease   The sites in the peripheral nervous system, however, have been chosen for this introduction to chemical pharmacology because of their relative simplicity

When the actions of drugs directly on tissues are considered, it is found that some of these are very specialized   Some compounds, such as certain sex hormones, act only on particular organs and are usually regarded as a problem in biochemistry   Other compounds, however, are much less specialized in their actions and substances which act like, or antagonize the actions of, histamine have been selected as examples of drugs acting directly on smooth muscle-cells

Drugs can modify the actions of cells in a great variety of ways   Local anaesthetics, for example, will suppress the action-potential in a muscle-fibre, just as they do in a nerve-fibre, although the muscle-fibre is usually much less sensitive and higher concentrations of the drug are needed to produce an effect   The actions of histamine, however, appear to involve only a small part of the cell surface (page 4) and to occur at receptors on the cell, but these histamine receptors seem to be quite different from those affected by acetylcholine (see Chapter VII)   Substances are known which will block the actions of histamine without affecting the actions of acetylcholine and it seems likely that there are, in fact, many different mechanisms and different receptors by which drugs can affect muscles or organs

334

Occurrence of Histamine

Histamine was synthesized by Windaus and Vogt (1907), because of its resemblance to the natural product pilocarpine (page 206) and the amino-acid histidine It was subsequently shown to be produced from histidine by bacterial decomposition (Ackermann, 1910) and to occur in ergot (Barger and Dale, 1910)

In man, and in all mammals, histamine is stored along with heparin in 'mast' cells (review by Riley, 1960) These are cells which are distinguished by their high content of granules, and these were originally thought to be composed of nutriment, hence the name 'mast', indicating fodder These granules, however, appear actually to be composed of histamine, which is basic, associated with heparin, which is acidic, and have a very high affinity for basic dyes, presumably because the histamine is displaced by the basic dye Mast cells are found throughout the connective tissue, particularly in the skin and in the capsular membranes surrounding organs, where they lie close to the small arterioles and capillaries In the blood the basophil leucocytes (white cells) may be regarded as mast cells, in addition, histamine is present in platelets In the platelets the histamine appears to be associated
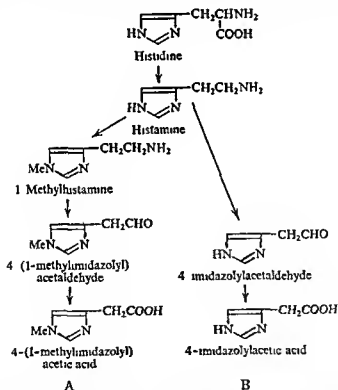
FIG X 1   *Formation and metabolism of histamine after the administration of a dose of isotopically labelled histamine to man the following proportions of labelled material were obtained from the urine histamine, 2–3 per cent methylimidazolyl acetic acid (A), 42–47 per cent imidazolylacetic acid (B) 9–11 per cent 1 ribosyl imidazol-4-acetic acid 16–23 per cent (Schayer, 1959)*

with adenosine triphosphate and diphosphate instead of with heparin The histamine found in the mast cells is formed in the body and is not obtained by absorption from the diet If histamine is given by mouth, the small amount which is absorbed from the gut is excreted in the urine, some unchanged, but the greater part as imidazolylacetic acid This could be formed by oxidative deamination of histamine by diamine oxidases, but it appears that in man the histamine is first methylated before oxidation (Fig X I, Schayer, 1956, 1959, 1960)

Actions of Histamine

Amounts of histamine of the same order as those found in the tissues can produce the following effects

1 Contraction of smooth muscle, such as that of the gut, bronchial tree, and uterus, this will lead, for example, to restriction of the passage of air into and out of the lungs

2 Relaxation of the smooth muscle of the arterioles and consequent dilatation, this will lead to a fall in blood-pressure

3 Increased permeability of the walls of the capillaries so that more of the constituents of the plasma can escape into the tissue spaces, this will lead to oedema

4 Increased secretion by many glands, such as the acid secreting glands of the stomach, the mucus secreting glands of the respiratory passages, and the tear glands

Release of Histamine from Tissues

Histamine may be released from the stores in the body when it is subjected to various kinds of maltreatment, such as mechanical damage or burning It may be physiologically active in removing the products of cell damage in the ordinary hazards of existence and, indeed, mast cells are particularly

48/80, mixture of di, tri and tetramer.
X I

numerous in the skin of those parts of the body most liable to minor trauma, e g feet, snout, and ears Histamine may also be liberated by drugs (review by Paton, 1957), one of the most effective being the substance *48/80* (X I) The most serious and most common circumstance in which histamine is liberated is in allergic conditions, such as the urticarial rashes of the skin the nasal distress of hay fever, the respiratory distress of asthma, and the general fall in blood pressure and severe constriction of the bronchi in anaphylactic shock

Tissues become hypersensitive (allergic) when antibody becomes fixed on the cells Antibody is the term given to specific substances (γ globulin, and, in some cases, β globulins) produced by lymphoid tissue when foreign proteins (antigens) enter the body Substances which are not proteins (haptens) can also lead to the formation of antibody by combining with body proteins to produce a complex which the lymphoid

tissue treats as if it were a foreign protein The lymphoid tissue forms antibody specifically to fit the template provided by the antigenic foreign protein (Fig X 2), and the production of antibodies is a very important part of the defence mechanism against invading micro-organisms In the case of a bacterial infection, antibodies are produced against the toxic metabolic products of the bacteria (the bacterial toxins) and against the proteins of the bacterial cell These antibody globulin molecules have two sites which have a configuration such that they will fit, and combine specifically with, the particular antigen which caused their production They therefore unite with the toxin and remove its toxic properties by incorporating it in an aggregate (Fig X 2), which is then taken up and degraded by phagocytic cells Antibodies to the proteins of the bacterial cell wall combine with it and cause a disorganization of the organism so that it does not reproduce and is more easily engulfed and destroyed by phagocytes
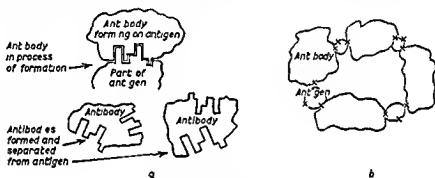
FIG X 2

a Formation of antibodies
b Combination of antibodies with antigen

Note *Antigen may be polyvalent antibodies are divalent*

Unfortunately any foreign protein, not only those which are harmful, will cause production of antibodies Very large amounts of antibody may, therefore, be formed when dust, mould spores, pollen, or animal scurf enters through the nasal passages, lungs, or even *via* the eye, and also when un denatured food enters through the gut wall, should this be abnormally permeable

Antibodies are freed from the lymphoid tissue into the blood stream, but a proportion quite quickly becomes adsorbed on, and may be incorporated in, the surface of cells It is not certain whether this adsorption is on all cells or only on certain types, but it is known that areas of inflammation take up more than normal tissues If the tissue is sensitized, i e if sufficient antibody is attached to the cells, a further exposure to antigen and the consequent combination of antibody with antigen, appears to activate enzymes which lead to the release of histamine and other pharmacologically active agents An allergic reaction will not take place if so much antibody is produced that combination with antigen occurs in the blood stream or only involves a small proportion of the tissues, or if antibodies of a different type are produced

which combine with antigen, but are believed not to be fixed on tissues It will also fail to occur if only small amounts of antibody have been produced, as would happen if the antigen were poorly absorbed into the body or was rapidly broken down

Uses of Histamine and Antagonists of Histamine

Histamine is given subcutaneously in man when it is necessary to test the ability of the stomach to produce hydrochloric acid As this action is not antagonized by antihistamine drugs, whereas the other actions of histamine are, one of these drugs is administered simultaneously to counteract the unpleasant vasodilatation, headache, and fall in blood-pressure which would otherwise occur Histamine has also been used in the diagnosis of phaeochromocytoma, a tumour of the adrenal medulla It causes the liberation of *nor*-adrenaline and adrenaline from this tumour and consequently the blood-pressure rises instead of falling, as it would in a normal subject

Substances which antagonize the actions of histamine are used to suppress the symptoms produced by the release of histamine in allergic conditions They may be very effective in the treatment of skin rashes or hay fever and in anaphylactic shock, but are not particularly effective in the treatment of asthma The reason for this may be that in this condition other substances which cause constriction of the bronchi are released besides histamine

Tests for Histamine-like and Antihistamine Activity

As histamine causes contraction of most smooth muscle, the actions of histamine-like compounds and of antihistamines may readily be studied on the isolated guinea pig ileum (page 144) or on the guinea pig uterus As it causes a dilatation of blood-vessels, the effects may be tested in the anaesthetized or spinal cat (page 191), the fall in blood pressure indicating an action like histamine To prevent effects at acetylcholine receptors, which would also produce a contraction of the guinea pig ileum or uterus, or a fall in blood pressure, it may be necessary to perform these experiments in the presence of atropine

To study the effects on bronchial tissue, perfused lung preparations may be used (page 291), or the substance may be tested for its ability to produce, or prevent, bronchospasm in intact guinea pigs The substance causing spasm is administered as a spray and the protection conferred by a particular dose of antihistamine (injected prior to exposure) can be estimated by comparing the duration of exposure which causes an animal to collapse from anoxia after treatment with the antihistamine, with the duration in the same animal before treatment

Effects on the permeability of cells, particularly those in the skin, may be studied by injecting a suitable dye (such as Coomassic Blue) which will penetrate and stain those tissues whose permeability has been increased by histamine In man there is no need to use a dye, compounds may be tested for their ability to produce a weal and flare (see, for example, Bain 1951)

Effects on acid gastric secretion may be studied in anaesthetized animals

in which the stomach is perfused, **or in conscious man by means of a stomach tube**

With all these preparations the activity of an antagonist could be expressed as the affinity constant, if it acts competitively, and the activity of an agonist as an equipotent molar ratio relative to histamine In order to obtain an indication of the ability of a compound to control allergic symptoms, material from sensitized animals may be used and the contractions (or vasodilatation) produced by addition of antigen instead of by addition of histamine

AGONISTS

Compounds Related to Histamine

Histamine is by far the most active of the 4 (aminoalkyl)imidazoles Pyman (1911) made the aminometyl and 3 amino-*n*-butyl compounds (X 2 and 3) and quotes the otherwise unpublished observations of Laidlaw that these are only feebly active Akabori and Kaneko (1936) found that the 3 amino-*n*-propyl compound (X 4) was quite active, but not as potent as histamine

$$HN{\underset{\displaystyle N}{}}{\diagup}{\overset{\displaystyle R}{}}$$

R = —CH$_2$NH$_2$, X 2,
 = —CH$_2$CH$_2$CHMeNH$_2$, X 3,
 = —CH$_2$CH$_2$CH$_2$NH$_2$, X 4,
 = —(CH$_2$)$_4$NH$_2$, X 5,
 = —CH$_2$CHMeNH$_2$, X 6,
 = —CHOHCH$_2$NH$_2$, X 7,
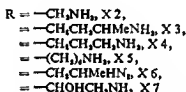
and that the 4 amino-*n*-butyl compound (X 5) was virtually inactive The α-methyl derivative, 4-(2 amino-*n* propyl)imidazole (X 6) was studied by Alles, Wisegarver, and Shull (1943) and, although it is not oxidized by diamine oxidases, it is not a particularly potent substance The equipotent molar ratio relative to histamine was 100 on the dog blood pressure, 100 to 300 on guinea-pig intestine, and 200 for effects when injected intradermally in man The hydroxy compound, 4-(2 amino-1-hydroxyethyl)imidazole (X 7), was prepared by Pyman (1916), who quotes the otherwise unpublished report of Dale that this compound, though active, is less potent than histamine

Me—⎡⎯CH$_2$—CHR—NH$_2$
HN$\diagdown$N

R = H, X 8,
R = Me, X 10

Me—⎡⎯CH$_2$—CHR—NH$_2$
N$\diagdown$NH

R = H, X 9,
R = Me, X 11
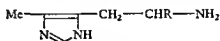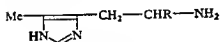
The 4- or 5-methyl derivative (4-methyl-5-aminoethylimidazole, X 8, or 5-methyl-4-aminoethylimidazole, X 9), with the methyl group adjacent to the aminoethyl group, was reported by Ewins (1911) to be less active than histamine and Alles, Wisegarver, and Shull (1943) found the equipotent molar ratio relative to histamine to be 100 on the dog blood-pressure, 100 to 300 on guinea pig intestine, but as low as 50 when tested intradermally in man

The corresponding α methyl derivative (4-methyl 5 (2 amino n propyl)imid azole, X 10, or 5 methyl-4-(2 amino n propyl)imidazole, X 11) was even less active on the dog blood pressure and guinea pig intestine but appeared to have the same activity when tested intradermally in man In this test, there-fore, but only in this test, it appears to be more active (equipotent molar

Me—[imidazole ring]—CH₂—CH₂—NH₂
X 12
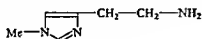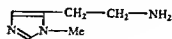
[imidazole ring, N—Me]—CH₂—CH₂—NH₂
X 13

ratio relative to histamine, 50) than the compound without the extra methyl group, α methylhistamine (X 6), for which the ratio was 200

Both 1 methyl and 3 methyl histamine (X 12 and 13) appear to be feeble or inactive (Pyman, 1916, Lee and Jones, 1949, Table X 1), but 2 methyl-histamine (X 14) has some activity (Tamamushi and Shibota, 1934, Lee and

HN—[imidazole ring, Me]—CH₂—CH₂—NH₂
X.14
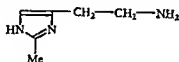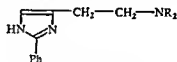
HN—[imidazole ring, Ph]—CH₂—CH₂—NR₂
R = H X 15,
R = Me X 16

Jones, 1949) 2 phenylhistamine (X 15) and 2 phenyl-4-dimethylamino-ethylimidazole (X 16) also appear to have some activity (Huebner, 1951) 1 Aminoethylimidazole (X 17) is only feebly active (Pyman 1916)

Derivatives of histamine, in which the nitrogen atom in the side chain is alkylated were prepared by Garforth and Pyman (1935) and studied by Vartiainen (1935) and similar compounds have been prepared and tested by

H₂N—CH₂—CH₂—[imidazole ring]
X 17
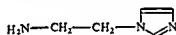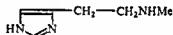
[imidazole ring]—CH₂—CH₂NHMe
X.18

Huebner, Turner, and Scholz (1949) The results (Table X 2) indicate that substitution of alkyl groups in this side-chain amino group leads to a decline in histamine like properties on the cat's blood pressure The N methyl compound, 4-(methylaminoethyl)imidazole (X 18), however was more active than histamine when tested on guinea pig intestine (equipotent molar

HN—[imidazole ring]—CH₂—CH₂—ṄMe₃
X 19
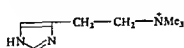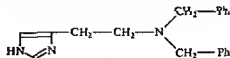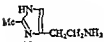
HN—[imidazole ring]—CH₂—CH₂—N(CH₂—Ph)(CH₂—Ph)
X.20

ratio, 0 5 Schild 1947, obtained a ratio of 0 4) but higher homologues were less active The N isopropyl derivative of histamine was also prepared by Sheeban and Robinson (1949), who quoted an equipotent molar ratio of 30 relative to histamine for its effects on the dog blood pressure

The quaternary methio-compound (X 19) was devoid of histamine like activity but had nicotine like properties The N dibenzyl derivative (X 20)

TABLE X 1

Histamine-like Properties of Analogues of Histamine

	Equipotent molar ratio relative to histamine on	
	Cat blood pressure	Guinea pig intestine
HN⎯⎯N Me⎯ ⎯CH₂CH₂NH₂	6 7	3 3
Me N⎯ ⎯CH₂CH₂NH₂	500	170
⎯N ⎯CH₂CH₂NH₂	12	3 3
⎯N ⎯CH₂CH₂NH₂	170	50
S Me⎯ ⎯CH₂CH₂NH₂	50	6 7
Me N⎯ ⎯CH₂CH₂NH₂	330	200
N⎯ ⎯CH₂CH₂NH₂	50	11 *
N⎯ ⎯CH₂CH₂NH₂	50	20
N⎯ ⎯CH₂CH₂NH₂	500	110
N⎯N ⎯CH₂CH₂NH₂	670	500

* Arunlakshana and Schild (1959) obtained ratios of 30–60

Lee and Jones (1949)

had no histamine-like activity but was a weak antagonist of histamine and Huebner, Turner, and Scholtz found that some members of a similar series of dialkylaminomethylimidazoles (X 21) were feeble antihistamines, although

$$\text{HN}⎯⎯\text{N} \quad \text{CH}_2⎯\text{NRR'}$$

NRR' = NMe₂, NEt₂, N◯, NMeCH₂Ph,
NEtCH₂Ph, N(CH₂Ph)₂, X 21
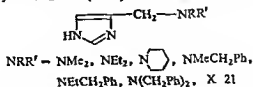
others were feebly histamine-like They also observed that β naphthyloxy-ethylimidazole (X 22) had appreciable activity, although the effects produced were only transient

$$\text{HN}⎯⎯\text{N} \quad \text{CH}_2⎯\text{CH}_2⎯\text{O}⎯⎯◯◯$$

X 22
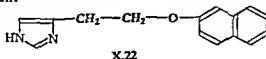
TABLE X.2
Histamine like Properties of Analogues of Histamine

	Equipotent molar ratios relative to histamine on		
	Cat blood-pressure	Guinea pig intestine	
	(V)	(V)	(H)
R =			
NHMe	20	0 5	—
NHEt	20	Large	1 3
NH*iso*Pr	—	Large	1 3
NMe₂	5–10	3 0	1 3
⁺NMe₃	Large	Large	—

V = Vartiainen (1935) H = Huebner, Turner, and Scholz (1949)
Other compounds examined by the latter and found to be inactive were those in which
R was NHPr, NEt₃, NPr₂, N◯, N◯O, NMeCH₂Ph, NEtCH₂Ph, and OPh

Effects of Altering the Imidazole Ring

Compounds in which the imidazole ring has been replaced by other hetero-cyclic structures have been examined by Lee and Jones (1949) on the intestine and blood pressure, and by Grossman, Robertson, and Rosiere (1952) on gastric secretion The results (Table X 1) indicate that histamine-like activity is confined to molecules very similar to histamine Sheehan and Robinson (1949), for example, tested 4-aminoethyl-1 2 3-triazole (X 23), a triazole
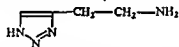
X.23

analogue of histamine, and found it was a weak antagonist of the effects of histamine on the dog blood-pressure This compound was also one of the very few substances which antagonized the effects of histamine on gastric secretion Most of the compounds related to histamine appeared to have little effect on secretion, though some caused an increase Of these, 4-(amino-ethyl)thiazole (X 24), 3 (aminoethyl)pyrazole (X 25), 5-methylhistamine

X.24

X.25
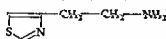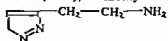
(X.8), α-methylhistamine (X 6), and 5 methyl-α-methylhistamine (X 10) appeared to be the most active Stimulation was also produced to some extent by N-benzyl- and N-acetyl histamine (X 26 and 27)
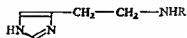
R = —CH₂Ph, X 26,
  = —CO—CH₃, X.27

Relationships Between Structure and Histamine-like Activity

Lee and Jones (1949) have discussed the structural features necessary for histamine like properties and have suggested that the most important part was the unit

$$=C\text{-}CH_2CH_2N=$$
$$=N$$
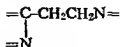
where the conjugated system was part of a small aromatic nucleus The nature of the nucleus was considered to be critical, hydrogen bonding might be important in the formation of the complex with the receptors

Histamine is a strong base with a pK_a at 30° C of 9 70 for the side-chain amino group and of 5 90 for the imidazole amino group (Levy, 1935) At body pH, therefore, it will be present almost exclusively as the univalent cation, with traces of the divalent cation Niemann and Hays (1942) have suggested that, of the two tautomeric forms of this univalent ion, one will be stabilized by resonance (Fig X 3), a hydrogen atom of the side chain amino
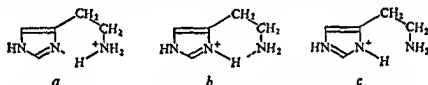
FIG X 3 *The structures a, b, and c, are possible for the histamine ion, which should be a resonance hybrid to which b and b should contribute most*

group being bonded to the tertiary nitrogen atom in the ring If the nitrogen at this position were secondary, as in the other tautomer, the hydrogen atom would lie out of the plane of the ring and would be less likely to interact with the side-chain amino group Niemann and Hays suggested that this ability to form an intramolecular hydrogen bond might be associated with histamine like activity Lee and Jones (1949) found that many compounds which could do this were inactive, although none of the active compounds could not form such a bond, 1 methyl-4-aminoethylimidazole (X 12), for example, had some weak histamine like activity (Table X 1) in spite of Pyman's report that it was inactive (page 350) but 1 methyl 5 aminoethyl imidazole (X 13) had no activity at all Jocelyn (1957) prepared the 2 amino-ethyl-3 methylimidazole (X 28), which can form such a bond, but this was

X.28
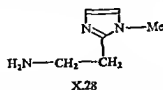
also inactive It may be questioned whether intramolecular hydrogen bond formation is essential for ability to act like histamine and is not rather merely associated with it

histamine, but F 929 was much more active (Bovet and Staub, 1937), and the replacement of the ether oxygen atom by an amino group led to the discovery of other antihistamine drugs, of which the most potent was F 1571 (X 34, Table X 3, Staub, 1939) This work led to the discovery of two types
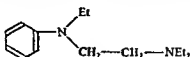
F1571, X34

of potent antagonist of histamine, substituted ethylene diamines related to F 1571 and dialkylaminoalkyl ethers related to F 929 Ability to antagonize histamine has subsequently been found in four other main types of compound, phenothiazine derivatives, piperazine derivatives azafluorene deriva

TABLE X 3

Antagonism of the Actions of Histamine by Phenylalkamine Ethers and N Phenylethylene Diamines

		Effects on	
		Whole guinea pigs anti histamine index	Isolated guinea p g intestine equipotent molar ratio
F 928	PhOCH CH NEt₂	1	1–5
F 929	Me⟨⟩OCH₂CH₂NEt₂ / CHMe₂	3	0 04–0 08
F 1379	Me₃HC⟨⟩OCH₂CH₂NEt₂ / Me	3	0 04–0 08
F 1482	Me⟨⟩OCH₂CH₂NHEt / CHMe₂	1	0 3
F 1571	PhNEtCH CH NEt	4	0 045–0 09
F 1335	PhNMeCH₂CH NEt	1 5	0 4
F 1167	PhNHCH₂CH₂NEt⁺	1	1 0*
F 1691	MeMe₂C⟨⟩NHCH₂CH₂NEt₂	3	0 08–0 15
F 1699	MeHC⟨⟩NEtCH₂CH₂NEt₂ / Me	1	0 14
Papaverine		1	1 1

The antihistamine index for whole guinea pigs is the number of lethal doses of histamine required to kill the animal after it had received 5 mg of the compound The equipotent molar ratio on the isolated intestine is expressed as the number of molecules of the compound required to produce the same degree of antagonism of histamine as one molecule of 1167 (marked with an asterisk)

Staub (1939)

lives, and propylamines and related compounds developed from antagonists of acetylcholine. For comparison, the pA_2 values for a number of compounds on the guinea-pig ileum are shown in Table X.4.

TABLE X 4
Antagonists of Histamine:
A_2 Values on Isolated Guinea-pig Ileum
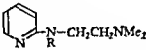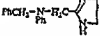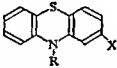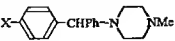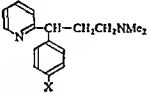
		pK_a, 20°	pA_2 (10 min)
Ethylene diamines:			
R = MeO	Mepyramine (14')(15')	8 85	9 36, 8 95* 9 46 (S) 9 33 (Re)
	Tripelennamine	8 95	9 00, 8 49*
	Methapyrilene	8 85	8 63
Cl	Chloropyrilene	8 70	9 50
Br	Bromopyrilene	8 63	9 64
$PhCH_2-N-H_2C$	Antazoline (15')	10 00	7 67 (7 40) 7 67 (Re)
Ethers:			
$Ph_2CHOCH_2CH_2NMe_2$, Diphenhydramine (14')		8 98	8 14, 7 68* 8 02 (S)
Phenothiazines:			
X = H; R = $CH_2CHMeNMe_2$, Promethazine (14')(15')		9 08	8 93 9 21 (E) 9 18 (E)
X = H; R = $CHMeCH_2NMe_2$, IsoPromethazine (14')			8 33 (E)
X = Cl; R = $CH_2CH_2CH_2NMe_2$, Chlorpromazine (14')			7 87 (R)
Piperazines:			
X = H, Cyclizine		8 16	7 87
X = Cl, Chlorcyclizine		8 15	8 63, 8 80* (7 98)

TABLE X 4—*Continued*

	pK$_a$, 20°	pA$_2$ (10 min)
Azafluorene derivatives		
Phenindamine (X.69)	8 98	8 46
Nu 1326 (X.70)	7 71	6 39
Nu 1525 (X.71)	8 66	5 71
Propylamine derivatives		
X = H, *Pheniramine*	9 23	7 82
X = Cl, *Chlorphenlramine*	9 16	8 82

X = H, *Pheniramine*

X = Cl, *Chlorphenlramine*

Unless otherwise indicated the results are those of *Marshall* (1955) Values marked with an asterisk were obtained when the experiments were performed in the presence of atropine and the values in parentheses are for different samples of the same drug The results were the mean of at least four determinations and the standard error is of the order of 0 2 of a unit so there is fairly good agreement between the figures In these experiments the antagonism was assessed after the drug had been in contact with the tissue for 10 minutes In the experiments of other workers also included in this table the drug was allowed to act for a longer period (14 or 15 minutes as indicated)

$$E = Edge\ (1953) \quad R = Ryall\ (1956) \quad Re = Reuse\ (1948) \quad S = Schild\ (1947)$$

Substituted Ethylene Diamines

The compound *Antergan* (X 35, Halpern, 1942), developed from *F 1571*, was much more active than it, but even greater activity was found in α pyridyl derivatives, such as Mepyramine (*Neoantergan*, *Anthisan*, X 36, Bovet,

Antergan, X 35
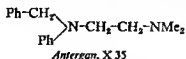
Horclois, and Walthert, 1944) The former, when given in the maximum dose which could be tolerated, protected guinea pigs against 50–60 lethal doses of histamine, whereas the latter, in a dose of 1 mg/kg (considerably less than the maximum tolerated) protected them against 75 lethal doses of histamine The desmethoxy compound, Tripelennamine (*Pyribenzamine*, X 37, Huttrer, Djerassi, Beears, Mayer, and Scholz, 1946), though less active than Mepyramine (Table X 4), also appeared to be potentially useful (Yonkman, Chess, Mathieson, and Hansen, 1946) The α pyridyl group was extremely important, both β- and γ-pyridyl derivatives were less active but 2 pyrimidyl derivatives, such as *Thonzylamine* (*Neohetramine*, X 38) had

appreciable activity (Feinstone, Williams, and Rubin, 1946, Aaron and Criep, 1948)

The benzyl group may be replaced by 2-thienyl, as in the compound *Methapyrilene* (X 39), and in these compounds, as in the benzyl analogues,
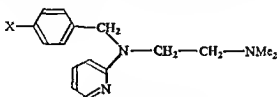
X = MeO, Mepyramine, X 36,
= H, Tripelennamine, X 37,
= Br, X 40,
= Cl, Halopyramine, X 43

activity may be increased by the introduction of a halogen atom Litchfield, Adams, Goddard, Jaeger, and Alonso (1947) found the drug ratio (page 43) for histamine and Tripelennamine on the guinea-pig ileum to be 25, whereas for the *p*-bromo compound (X 40) it was 50, and in the same experiments the
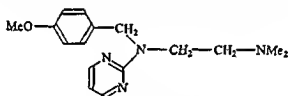
Thonzylamine, X 38

drug-ratio for histamine and *Methapyrilene* was 15, whereas for the chloro and bromo compounds (*Chloropyrilene* and *Bromopyrilene*, X 41 and 42) it was 100 The very high activity of these compounds on the guinea-pig ileum is also shown in Table X 4, but their activity *in vivo* does not appear to be quite so outstanding The *p* chloro analogue of Mepyramine and Tripelenn-
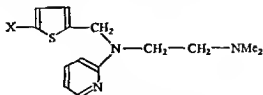
X = H *Methapyrilene*, X 39,
= Cl, *Chloropyrilene*, X 41,
= Br, *Bromopyrilene*, X 42

amine, *Halopyramine* (*Synopen*, X 43) appears to be very similar to the *p*-bromo compound (X 40) and to Mepyramine, Gatzek and Mechelke (1950), for instance, found that comparable degrees of antagonism of the effects of histamine on the blood-pressure in man were produced by 0 5 mg Mepyramine, 1 mg *Halopyramine*, and 15 mg Tripelennamine

The compound Antazoline (X 44, Meier and Bucher, 1946), which is an imidazoline whose structure resembles that of histamine (on paper) can also be regarded as belonging to this type of compound It is not particularly active (Table X 4)

In compounds of this type high activity appears, in general, to be associated with a dimethylaminoethyl group rather than any other side-chain The α methyl analogue of Mepyramine (X 45) and the dimethylamino-n propyl analogue (X 46) are much less active (Bovet, 1947)
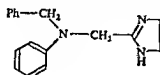
Antazoline, X.44
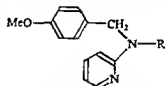
R = CH₂CHMeNMe₂, X.45
= CH₂CH₂CH₂NMe   X 46

Dialkylaminoalkyl Ethers

Many compounds of this type, which can be regarded as developments from F 929, were studied by Loew, Kaiser, and Moore (1945) The most active was the compound Diphenhydramine (*Benadryl*, X 47) which has been used clinically, although it is not as active as Mepyramine and often produces unpleasant side effects (particularly depression of the central nervous system) In this series, the structure of the basic side-chain does not appear to be so critical (Table X 5), but activity is enhanced by the introduction of a

TABLE X.5

Antagonism of the Effects of Histamine on Intact Guinea pigs by Compounds Related to Diphenhydramine

	Equipotent molar ratio relative to Diphenhydramine
Ph₂CHOCH₂CH₂N	0 88
Ph₂CHOCH₂CH₂N	1 8
Ph₂CHOCH₂CH₂NHMe	4 3
Ph₂CHOCH₂CH₂N·NEt₂	7 5
F 1571	2 3
F 929	4 2

Loew, Kaiser, and Moore (1945)

p-methyl group, as in *Toladryl* (X 48), or a p-bromo group, as in *Ambodryl* (X 49) McGavack et al (1950 1951) reported that these compounds would protect guinea pigs against the effects of a histamine aerosol in doses between one half and one quarter of those of Diphenhydramine, and similar results have been obtained by Ensor, Russell and Chen (1954)

The α-pyridyl derivative, *Doxylamine* (*Decapryn*, X 50, Sperber, Papa, Schwenk, and Sherlock, 1949) also appears to be more active than Diphenhydramine itself, Lovejoy, Feinberg, and Canterbury (1949) found it to have activity comparable with Tripelennamine and Mepyramine in suppressing the flare produced by histamine in human skin (see also Table X 10)
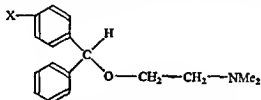
R = H, Diphenhydramine, X 47,
= Me, *Toladryl*, X 48,
= Br, *Ambodryl*, X 49

Although all the antihistamine drugs so far discussed have been tertiary bases, it is interesting that quaternary salts are not necessarily inactive Loew, MacMillan, and Kaiser (1946) found that the quaternary methoderivative of Diphenhydramine (X 51), though less active than the tertiary base, produced a comparable antagonism of the effects of histamine on the
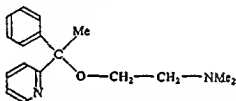
Doxylamine, X 50

guinea pig ileum when given in about three times the concentration This suggests that tertiary bases are likely to be acting as the ions, competing with histamine ions, which is consistent with their high basicity (Table X 4) The absence of quaternary ammonium salts with antihistamine properties which can be made use of clinically can be ascribed to the difficulty with which
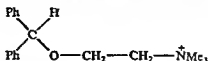
X 51

such compounds would be absorbed from the stomach and intestine, and to the likelihood of their possessing other actions, particularly ability to antagonize acetylcholine The quaternary metho-derivative of Diphenhydramine, for instance, is the benzhydryl ether of choline and has marked atropine-like activity

Phenothiazine Derivatives

Halpern and Ducrot (1946) found extremely high antihistamine activity in the phenothiazine derivative Promethazine (*Phenergan*, *Avomine*, X 52),

which conferred a degree of protection on guinea pigs several hundred times that produced by *Antergan* and about a thousand times that produced by *F 929* Many phenothiazine derivatives have been made (review by Viaud, 1954), and antihistamine activity appears to be greatest in Promethazine and in the dimethylamino compounds closely related to it (Table X 6) It is interesting that the quaternary metho derivative of Promethazine (X 53), like the quaternary derivative of Diphenhydramine, has appreciable antihistamine activity, though less than that of the parent tertiary base
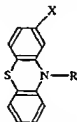
X = H , R = CH₂CHMeNMe₂ , *Promethazine* , X 52 ,

X = H , R = CH₂CHMeNMe₃ , X 53 ,

X = H , R = CH₂CH₂NEt₂ , *Diethazine* , X 54 ,

X = Cl , R = CH₂CH₂CH₂NMe₂ , *Chlorpromazine* , X 55 ,

X = H , R = CH₂CH₂N⟨ ⟩ , *Pyrathiazine* , X 56 ,

X = H , R = CH₂—⟨ ⟩ , *Methdilazine* , X 57 ,

X = H , R = CH₂—⟨ ⟩ , *Pacatal* , X 58 ,

X = H , R = CH₂—⟨ ⟩ , X 59 ,

X = H , R = ⟨ ⟩NMe , X 60 ,
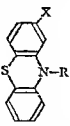
X = H , R = CH₂—⟨ ⟩NMe , X 61

Promethazine has been resolved by Toldy, Vargha, Toth, and Borsy (1959) and the (+)- and (−)-forms appear to have the same antihistamine activity, toxicity, and activity on the *central nervous system* (Borsy, Lazar, Csizmadia, and Toldy, 1959)

The diethylaminoethyl compound, *Diethazine* (X 54), is much less active and, like *Chlorpromazine* (X 55), is of interest primarily because of its effects on the central nervous system The pyrrolidinoethyl compound *Pyrathiazine* (*Pyrrolazote*, X 56) does not appear particularly active in Table X 6, although Van der Brook *et al* (1948) found that in their experiments on guinea pig intestine it was as active as Tripelennamine The N-methyl-3-pyrrolidyl-methyl compound, *Methdilazine* (X 57), however, has been found to produce effects on guinea-pig intestine comparable with those produced by Promethazine in one-quarter of the concentration, or one-fifth of the concentration of Tripelennamine, or one hundredth of the concentration of Diphenhydr-

TABLE X 6

pA₂ Values of Phenothiazines on the Guinea pig Ileum

		pKₐ (20°)	pA₂ (10 min)
X = H, R = CH₂CH₂NMe₂	*3015 RP*	8 66	8 76
CH₂CH₂NEt₂	*Diethazine*	9 09	7 88 (7 95)
CH₂CH₂CH₂⬡	*Pyrathiazine*	8 96	7 81
CH₂CHMeNMe₂	*Promethazine*	9 08 (8 92)	8 93 (8 78)
CH₂CHMeNMe₂	*3554 RP*	—	8 18
CH₂CHMeNEt₂	*Ethopropazine*	9 50	8 34
CH₂CHEtNMe₂	*4605 RP*	9 02	7 60
CHMeCH₂NMe₂	*IsoPromethazine*	8 91	7 97*
CHMeCHMeNMe₂	*3349 RP*	9 61	6 50
CH₂CH₂CH₂NMe₂	*Promazine*	9 52	8 25
CH₂CMe₂CH₂NMe₂	*3300 RP*	9 13	7 00
X = Cl, R = CH₂CH₂NMe₂	*Chlorpromazine*	9 30	7 92*

The estimates of pA₂ were the mean of at least four experiments, the experimental error appears to be about 0 2 and the results in parentheses, which were obtained with other samples of the drug, are not significantly different The compounds marked with an asterisk were tested by Edge (1953) and Ryall (1956), whose results are shown in Table X 4 With some of the compounds equilibrium may not have been reached in the short time allowed (10 minutes)

Marshall (1955)

amine (Lish, Albert, Peters, and Allen, 1960) The analogous N methyl-3-piperidylmethyl compound, *Pacatal* (X 58), is essentially of interest because of its effects on the central nervous system, rather than as an antagonist of histamine (Nieschulz, Popendiker, and Sack, 1954, Nieschulz, Popendiker, and Hoffmann, 1955) and other compounds of this type, such as the 2 imidazolylmethyl derivative (X 59), and the N methyl-4-piperidyl and N-methyl-4-piperidylmethyl compounds (X 60 and 61), are likewise relatively feeble antagonists of histamine (Nieschulz, Popendiker, and Scheuermann, 1956, Nieschulz, Hoffmann, and Popendiker, 1956)

The central actions of these compounds are undesirable in an antihistamine and appear to be dependent, at least to some extent, on the presence of the phenothiazine nucleus The related 10-thia 1 9 diaza-anthracene derivatives appear to have much less effect on the central nervous system and to possess even greater antihistamine activity than the phenothiazines (Schlichtegroll, 1957) The relationships between structure and activity appear to be similar to those in the phenothiazines (Table X 7) and the analogue of Promethazine,

TABLE X 7

Antagonism of the Effects of Histamine by 10 thia 1 9 diaza anthracene Derivatives

	Equipotent molar ratios relative to the dimethylaminoethyl compound (R = $CH_2CH_2NMe_2$) in tests with	
	Whole guinea pigs	Guinea pig ileum
R =		
$CH_2CH_2NMe_2$	1	1
$CH\cdot CH_2NMe_2$	0 12	1 3
$CH\ CH_2NEt_2$	10	10
CH_2CH_2⬠	0 60	0 78
$CH\ CH_2$⬡	*ca* 35	28
$CH_2CH_2CH_2NMe_2$	3 8	2 1
$CH\ CH_2CH_2NEt_2$	350	16
CH_2CH_2N⬠	*ca* 3 9	10
$CH_2CHMeNMe_2$ (*Isothipendyl*)	0 31	0 85
$CH_2CHMeNMe_2$	0 31	2·9
$CH_2CHMeNEt_2$	*ca* 12	3 9
$CH\ CHMeCH_2NMe_2$	0 35	2 1
Antazoline	8 2	19

Activity Relative to Other Drugs on the Guinea pig Ileum

	pA_2 (2 mins)
Antazoline	7 15
Isothipendyl	8 34
Promethazine	7 76
Diphenhydramine	7 45
Mepyramine	8 41
Chlorpheniramine	8 05

Note – These pA_2 values obtained after only 2 minutes exposure to the drug, are much lower than those obtained after longer periods of exposure and may be greatly affected by differences in the rates of diffusion of the drug to the receptors in the tissues

Schlichtegroll (1957)

Isothipendyl (*Andantol Nilergex*, X 62) appears to be very effective clinically In this series some quaternary derivatives have very high activity in protecting guinea pigs against asthma produced by histamine, much higher than is consistent with their antihistamine activity on intestine (which is less than that of the parent tertiary bases) The tricyclic ring structure in these com

pounds is extremely important, N-phenyl-N-α-pyridyl-N'N'-dimethylethylene diamine (X 63), for instance, is very much less active even than Antazoline, at least fourteen times the dose is needed to produce a comparable degree of antagonism of the effects of histamine on the guinea-pig ileum

Isothipendyl, X 62

X 63
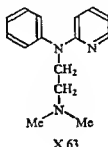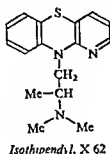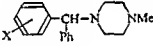
Piperazines

Many piperazines, particularly derivatives such as N-diphenylmethyl-N -alkyl-piperazines (X 64), have been prepared and found to have antihistamine activity (Cerkovnikov and Stern, 1946, Hamlin, Weston, Fischer, and Michaels, 1949, Castillo, De Beer, and Jaros, 1949) The most active compound of this type appears to be *Chlorcyclizine* (X 65, Tables X 4 and X 8)

TABLE X 8

Antihistamine Activity of Piperazine Derivatives pA₂ Values on Isolated Guinea-pig Ileum

X —⟨ ⟩—CH–N⟮ ⟯NMe Ph	pKₐ (20°)	pA₂ (10 mins)
X =		
H (*Cyclizine*)	8 16	7 87
p Br	7 97	8 15
p F	8 23	8 03
p Me	8 21	8 16
p-Cl (*Chlorcyclizine*)	8 15	8 63
m Cl	8 10	7 25

Marshall (1955)

The closely related substances, *Meclizine* (X 66) and *Buclizine* (X 67) are only feebly active in antagonizing the effects of histamine on the isolated guinea-pig ileum, but have a 'marked protective action' against the effects of a histamine aerosol on intact guinea-pigs (P An, Gardocki, and Reilly, 1954) Their effects are slow in onset and remarkably long lasting and are thought to be due to the action of metabolites formed from the compounds, rather than to the compounds themselves These piperazine derivatives have depressant effects on the central nervous system, like most (but not all) of

the other compounds which antagonize the effects of histamine, and some of them appear to be very effective in reducing vomiting The analogue of *Chlorcyclizine* without the chloro group, *Cyclizine* (*Marzine*, X 68), appears to be valuable for this purpose and is used for the prevention of travel sickness, its antihistamine activity, on the other hand, is relatively feeble (Table X 4, see also Norton *et al*, 1954)

X 64,
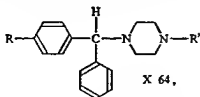
R = Cl   R' = Me, *Chlorcyclizine*   X 65,

R = Cl,   R = CH₂—⟨⟩—Me, *Meclizine*,   X 66,

R = Cl,   R' = CH₂—⟨⟩—CMe₃, *Buclizine*,   X 67,

R = H,   R' = Me, *Marzine*,   X 68

Azafluorene Derivatives

As has already been pointed out, the usefulness of many antihistamine drugs is limited by their effects on the central nervous system, especially by their liability to produce drowsiness This side effect is seen particularly with Diphenhydramine, but is produced also by all the early antihistamines The first indication that it was not necessarily associated with the ability to antagonize the effects of histamine was the discovery of the antihistamine
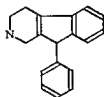
Phenindamine X 69
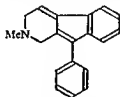
Nu 1326, X.70
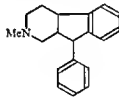
Nu 1525 X.71

activity of *Phenindamine* (*Thephorin*, X 69, Lehmann, 1948) This compound bears no obvious relation to the other antihistamine drugs known at the time when it was made It has moderate antihistamine activity (Tables X 4 and 9) and appears to stimulate the central nervous system, rather than to depress it The related compounds *Nu 1326* (X 70) and *Nu 1525* (X.71) are much less active, *Nu 1525* lacks the flatness associated with the indane portion of *Phenindamine* and *Nu 1326* is a much weaker base (Table X 4)

Antihistamine Activity of Phenindamine *and Related Compounds*

	Equipotent molar ratio relative to *Phenindamine* for antagonism of the effects of histamine on	
	Whole guinea pigs (histamine spray)	Isolated guinea pig ileum
Phenindamine	1	1
Nu 1326	—	60
Nu 1525	—	150
Diphenhydramine	2	2
Tripelennamine	0 5	0 3

Lehmann (1948)

Propylamines and Related Compounds

Many diphenylpropylamines, carbinolamines, and allylamines, prepared by Adamson (1949, Adamson and Billinghurst, 1950), were tested by White, Green, and Hudson (1951) for their ability to relieve the spasm of smooth muscle caused by a variety of agents, including acetylcholine (page 230) and histamine A moderate degree of antihistamine activity was found among many of these compounds, including some which were quaternary ammonium salts, but α-pyridyl derivatives were much more active (Green, 1953) The allylamines, *Triprolidine* (*Actidil*, 295 C 51, X 72) and 405 C 49 (X 73), and
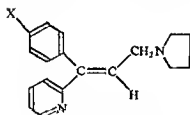
X = Me, *Triprolidine*, X 72,
X = Cl, 405 C 49, X 73
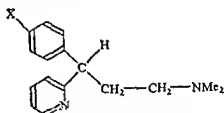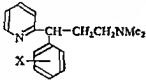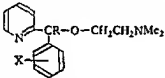
X = H, *Phenramine*, X 74,
X = Cl *Chlorphenramine*, X 75

the propylamines *Phenramine* (*Trimeton*, X 74) and *Chlorphenramine* (*Chlortrimeton*, X 75) appear to be particularly active, the equipotent molar ratios relative to Mepyramine for antagonism of the effects of histamine on the guinea-pig ileum were 0 6 for *Triprolidine*, 1 1 for 405 C 49, and 2 1 for *Chlorphenramine* *Phenramine* and *Chlorphenramine* have also been studied by Labelle and Tislow (1955) and their ability, when given orally, to protect guinea pigs against the lethal effects of injected histamine is shown in Table X 10

Chlorphenramine has been resolved and the optical isomers tested by Roth and Govier (1958) The (+) isomer is much more active than the (—)-,

TABLE X 10

Antihistamine Properties of α-Pyridylpropylamines

	Equipotent molar ratios relative to Diphenhydramine for protection of guinea pigs against effects of histamine (given intravenously)
X = H (*Pheniramine*)	0 43
= p-Me	0 082
= p-Cl (*Chlorpheniramine*)	0 020
and for some α pyridylmethyl ethers	
R = H, X = H	0 75
R = Me, X = H (*Doxylamine*)	0 39
R = Me X = p Cl	0 30

Note – The compounds were given orally, the equipotent molar ratios relative to Mepyramine should be ten to twenty times the values relative to Diphenhydramine shown here

Labelle and Tislow (1955)

estimates of the equipotent molar ratio for the racemate relative to the (+)-isomer were 2 3 and 2 4 in tests for ability to protect guinea-pigs against the effects of intravenously administered histamine, and 1 8 for protection against a histamine aerosol The ratio for the (−)-isomer relative to the (+)- was about 500

Somewhat similar stereospecificity appears to be observed with the allylamines These can exist in two geometrically isomeric forms, in which the nitrogen atoms of the basic group and pyridine ring are either *cis* or *trans*, and the active compounds, such as *Triprolidine*, have the *trans* configuration (Adamson, Barrett, Billinghurst, and Jones, 1957) These latter show absorption in the ultra-violet region characteristic of vinylpyridines, whereas the former show absorption characteristic of styrenes It appears, therefore, that in the *trans* compounds the double bond and pyridine ring are in the same plane and that the benzene ring is forced, for steric reasons, to lie at right angles to this plane (Fig X 5) Although the relationships between activity and the structure of the phenyl-α pyridylmethyl part of the molecule appear to be similar in both the allylamines and the propylamines, the effects of substituents on the basic group at the other end of the molecule are different,

the dimethylamino group was most effective in the propylamines (as it is with many antihistamines), but the pyrrolidino group was most effective in the allylamines (Adamson, 1951)
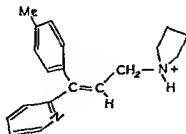
FIG X 5 *Structure of Triprolidine the pyridine ring and double bond are in the same plane, with the benzene ring forced at right angles to it*

Evidence for Competitive Antagonism

The antagonism of histamine by antihistamine drugs, such as those described above, is usually reversible and is assumed to be competitive Schild (1947) showed that the effects of Mepyramine on the dose-response curves for the guinea pig ileum were the same whether the contractions were produced by histamine or N methylhistamine (X 18), and Arunlakshana and Schild (1959) found the pA_2 value for Mepyramine and histamine on the guinea pig ileum was the same as that for Mepyramine and 2 (2 pyridyl)ethylamine (X 76), a similar result was obtained with Diphenhydramine (Table X 11, the pA_2 values of these compounds also do not appear to alter very greatly from one tissue to another) The fact that the pA_2 value is independent of the agonist, however, does not prove that the antagonism is competitive, only that the agonists are acting on the same receptors

X 76
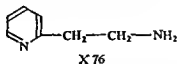
Marshall (1955) used the value of pA_2–pA_{10} as a test to see if antihistamines were acting competitively The reliability of this method is limited by the errors attached to the pA values, for competition, pA_2–pA_{10} = 0 95, whereas in a non competitive situation it is less than this (page 44) As the error attached to the pA values may be 0 2, the combined uncertainty in the value of pA_2–pA_{10} may make it difficult to show convincingly that the antagonism is, or is not, competitive Marshall's results however, suggested that all the common antihistamines (e g those shown in Table X 4) were acting competitively

The manner in which the compounds are acting, however, may not really be apparent unless a wide range of concentrations is studied Several β halo alkylamines have been found to have antihistamine activity (Nickerson and

TABLE X 11

Effect of Agonist on Blocking Activity of Mepyramine and Diphenhydramine at the Histamine Receptors of the Guinea pig Ileum

	pA$_2$ (14 mins) for contractions produced by	
	Histamine	2-(2 pyridyl)ethyl amine
Mepyramine	9 3	9 2
Diphenhydramine	7 9	8 0

Comparison of Blocking Activity at Histamine Receptors of the Guinea pig Ileum with those of Other Tissues

	pA$_2$ (14 mins) for	
	Mepyramine	Diphenhydramine
Guinea pig ileum	9 3	8 0
Guinea pig perfused lung	9 4	7 8
Guinea pig trachea	9 1	7 8
Human bronchi *	9 3	—

* Results of Hawkins and Schild (1951)

Arunlakshana and Schild (1959)

Harris, 1949), this does not appear necessarily to be associated with ability to block adrenergic receptors because the compounds which are very active at one site are not always very active at the other

Phenoxybenzamine (page 333) and *SY 28* (X 77) were the *most active*
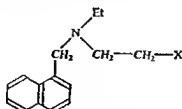
X = Cl or Br SY 28 X 77

antagonists of histamine, the latter, for example, antagonized the effects of histamine on intact guinea pigs and on the isolated guinea pig ileum in doses equal to, or not more than twice, those of Mepyramine When low concentrations of this substance were used, the antagonism was surmountable (page 16) and has been described as 'competitive' by Chen and Russell (1950) and Graham and Lewis (1953) When higher concentrations were used, however, it was observed to be unsurmountable Nickerson (1956) has

pointed out that these results (Fig X 6) are consistent with a non-competitive antagonism, similar to that observed at adrenergic receptors, in which the ethylene immunium ion reacts with the histamine receptors to form a stable complex The reason why the antagonism is surmountable when low concentrations are used appears to be that a maximal response of the tissue can be obtained by the combination of histamine with only a very small proportion of the histamine receptors, possibly with as little as 1 per cent In view of this evidence for the existence of 'spare' receptors on these tissues, it is difficult to decide whether antagonists of histamine are really acting competitively unless, for example, the graph of *dose ratio-minus one* against antagonist concentration is plotted over a wide range
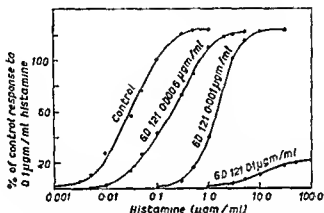
FIG X 6 *Evidence for non competitive action of SY-28 (GD 121, X 77) at histamine receptors and of the existence of 'spare' receptors, i e that a maximal response of the tissue does not involve a combination with all the receptors (Nickerson, 1956)*

Relationships Between Chemical Structure and Antihistamine Activity

Although more evidence would be welcome, it seems likely that most of the antihistamines are acting by competition with histamine for the receptors on smooth muscle cells Many of them are active in very low concentrations, about one hundredth of the concentrations of histamine which produce contractions (Arunlakshana and Schild, 1959, found the mean effective concentration of histamine in their experiments with the guinea pig ileum to be $10^{-7.7}$ M) The most active substances, with pA_2 values greater than 9, are Mepyramine, Tripelennamine, *Chloropyrilene* and *Bromopyrilene*, and Promethazine (Table X 4) *Isothipendyl* appears to be at least as active as Mepyramine and more active than Promethazine (Table X 7) and *Triprolidine* appears to have similar high activity (page 367)

These substances are all strong bases and changes in their chemical structure could conceivably lower activity by lowering their pK_a and reducing the proportion of ions available for competition with histamine ions That this is not the only effect of changes in structure, however, can be seen from

Table X 4 and from the other results of Marshall (1955), many compounds which are strong bases have only weak activity, though few of the compounds which are weak bases have strong activity

There is some similarity between the compounds listed above With the exception of Promethazine, they contain either the unit
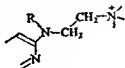
or the similar unit
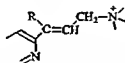
In Promethazine the unsaturated nitrogen atom is missing (it is present in *Isothipendyl*) and the group R is part of the phenothiazine nucleus This structure, however, is not flat but folded about the N–S axis (Fig X 7) and
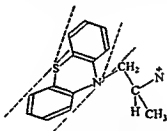
FIG X 7   *Promethazine*

there is, therefore, some resemblance to the other active antihistamines, this is even more marked in *Isothipendyl* The activity of the latter and the fact that *Triprolidine* has a *trans* structure suggest that the compounds are adsorbed with the groups arranged as in Fig X 8 The vinylpyridyl part of
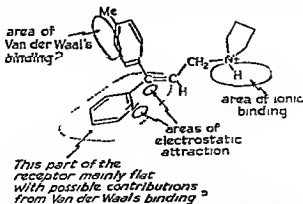
FIG X 8   *Hypothetical adsorption of* Triprolidine

Triprolidine must be flat and consequently the whole arrangement, except for the substituent, R, is likely to be more or less planar If this is correct, it follows that the active (+) form of *Chlorpheniramine* should have the R-configuration (Fig X 9) The points of attachment of this molecule would appear to be the onium group, the pyridine nitrogen atom and also the 3 carbon atom Some sort of attachment at the latter point would seem to be likely because the high stereospecificity of *Chlorpheniramine* indicates that
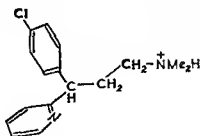
FIG X 9 R *Chlorpheniramine*

more than two points are involved and also because of the activity of compounds, such as Promethazine, which lack the pyridine nitrogen atom The pyridine nitrogen atom is drawn adsorbed in the position shown, rather than with the pyridine ring rotated through 180°, because *Isothipendyl* could not be adsorbed in the latter manner and also because in the position shown the three pharmacodynamic groups do not lie in line, with the ring rotated they would lie almost in line and there should be little stereospecificity The lack of stereospecificity in Promethazine is not inconsistent with this picture of the receptor, because both halves of the ring are equivalent and the fold in the molecule is not rigid With *Isothipendyl*, however, the two parts are not equivalent and it might, therefore, be expected that the isomers will be found to have different activities, and that the active isomer has the S-configuration
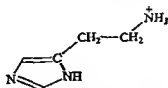
FIG X 10 *Tautomeric form of histamine, for comparison with Fig X 8 (but see text)*

The stereospecificity of molecules such as *Chlorpheniramine* could also arise, however, from adsorption of the part of the molecule containing the benzene ring If these were so, there would be no need to consider binding of the 3 carbon atom or tertiary nitrogen atom The effects on activity of substitution in the benzene ring could then be explained by supposing that substituents such as methyl, chloro, or bromo in the *p*-position increased affinity by Van der Waals' attraction to the receptors If there is no receptor

group binding this part of the molecule, these substituents may act by increasing the availability of electrons at the 3-carbon atom, at least in *Triprolidine* and *Chlorpheniramine*, by an electromeric shift and with the methyl group (but not chloro or bromo) possibly by an inductive effect

The unit common to active antagonists of histamine is similar to that which has been considered to be important for histamine-like activity (page 353) Gaddum (1948) pointed out that this unit

$$-\overset{|}{\underset{\parallel}{C}}-CH_2CH_2\overset{+}{N}$$

had its counterpart in Mepyramine and compounds related to it

$$=\overset{|}{\underset{\parallel}{C}}-N-CH_2CH_2\overset{+}{N}$$
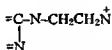
though the chain is longer by one nitrogen atom There would be greater similarity still if histamine were adsorbed at the receptor in the tautomeric form (Fig X 10, compare this with Fig X 8), but unfortunately this is not consistent with what has already been said about the adsorption of histamine and, in particular, with the observation that 1 methylhistamine (X 12) has some histamine-like activity, whereas 3 methylhistamine (X 13) has none If the antagonists are adsorbed because of the similarity pointed out by Gaddum, it might be expected that shorter compounds would be more active still

Differences Between Results Observed *in vivo* and Those Observed on Isolated Preparations

The ability to release histamine might be mistaken for histamine-like activity in tests on isolated preparations and this will also be true in tests on whole animals The time course of the response, however, should indicate what is happening, a true histamine-like response should be very rapid in onset, whereas a response caused by the release of histamine from the tissues should take a little time to develop This difference in time course should also be observed with substances which act by preventing the inactivation of histamine, although there are no compounds known, as yet, which definitely act in this way Inhibitors of histaminase (diamine oxidase), for instance, do not show any appreciable potentiation of the effects of histamine (Angelakos and Loew, 1957, 1958), and examples of compounds which block the methylation of histamine have not yet been discovered Compounds, such as α methyldopa (X 78), which prevent the formation of histamine by blocking histidine-decarboxylase (Sourkes, 1954) should be equally active *in vitro* and *in vivo* in reducing the effects of substances which act by releasing histamine, but should be ineffective in antagonizing the effects of histamine itself

The results of the *in vitro* tests of antihistamines may not give any real indication of their activity *in vivo*, particularly if the *in vivo* tests are based on ability to suppress allergic reactions rather than those of histamine itself In a test for ability to suppress the weal produced by histamine in human skin, for instance, Bain (1949, 1951) found that Mepyramine was much less active than would have been expected from the results of *in vitro* tests The equipotent molar ratios relative to Promethazine were 0 4 for *Chlorphenir-amine* and *405 C 49 (X 73)*, 2 4 for *Chlorcyclizine*, 5 2 for Mepyramine, 15 for Antazoline, and 20 for *Thonzylamine* When tested for ability to suppress urticaria clinically, the difference was even more marked, between 600 and 900 mg of Mepyramine were needed to produce the same control of the condition as was produced by 50 mg of Promethazine

α Methyldopa X 78
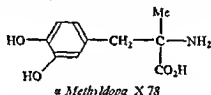
The greatest differences between the results of experiments on isolated tissues and those obtained in whole animals are the failure of antihistamines to suppress the action of histamine in stimulating the secretion of acid gastric juice and the failure to relieve most forms of asthma The failure to antagon ize acid gastric secretion is an advantage in the use of histamine to diagnose poor secretion of acid gastric juice in man, but it is a puzzling problem Possibly the receptors at which histamine is acting are so very dissimilar from other histamine receptors that antihistamines have no affinity for them That they are different is clear from the results discussed on pages 352 and 354, and perhaps the situation may be compared with the adrenergic receptors, at which the antagonists are quite sharply divided into those which will block α receptors but not β receptors, and those which block β receptors and do not block, or even stimulate, α receptors Another possibility is that hist amine does not itself stimulate gastric secretion but is metabolized into some compound which does Some evidence for this was obtained by Born and Vane (1953), who found that the response of the acid producing cells of the stomach to histamine was greater when the histamine was allowed to mix with blood for a short period before reaching the stomach The most likely metabolites, however, 1- and 3 methylhistamine (X 12 and 13), are without effect on gastric secretion (Grossman, Robertson, and Rosiere, 1952)

The failure of antihistamine drugs to antagonize the constriction of the bronchi which occurs in asthma suggests that this effect may be produced by other substances besides histamine Many substances will cause smooth muscle to contract, some of these are of relatively simple structure, such as 5 hydroxytryptamine (X 79) whereas others are of much more complex nature, such as polypeptides and complex derivatives of organic acids

The substance 5 hydroxytryptamine occurs in the gastro intestinal tract in platelets, and in the central nervous system It may be released from these

N

sites in certain circumstances (e g by the action of reserpine) and it is conceivable that the ability to antagonize 5 hydroxytryptamine as well as histamine might enhance the value of a drug in the control of allergic conditions The problem of antagonizing the actions of 5-hydroxytryptamine,

X 79
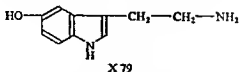
however, is difficult The most effective substances are lysergic acid diethylamide (*LSD*, X 80) and its 2 bromo derivative (*BromLSD*, *BOL*, X 81), and though these can be drawn to resemble 5 hydroxytryptamine (Robson and Stacey, 1962), they produce an unsurmountable block Even compounds quite closely related to 5 hydroxytryptamine, such as 5 benzyloxy N N dimethyltryptamine (X 82, Barlow and Khan, 1959), also appear to be acting

X = H, *LSD*, X 80
X = Br, *BromLSD*, X 81
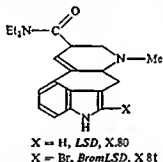
non-competitively and the relationships between chemical structure and antagonist activity are not easy to understand The compound 5 benzyloxy-N N dimethyltryptamine, for example, is a weaker antagonist than 5-benzyloxygramine (X 83, Gaddum, Hameed, Hathway, and Stephens, 1955), Barlow and Khan (1959) found the drug ratio for the latter to be about ten times that of the former For reviews of the actions of 5 hydroxy-

n = 2, X 82
n = 1, X 83
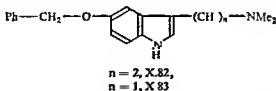
tryptamine and of compounds related to it, see Robson and Stacey (1962) and Gyermek (1961)

With the substances of more complex structure it is even more difficult to try to analyse the relationships between chemical structure and biological activity (for a review of pharmacologically active peptides, see Schachter, 1962) With the recent great advances in protein chemistry it has been possible to determine the constitution of a number of polypeptides, e g oxytocin and

vasopressin (Du Vigneaud *et al*, 1953, Tuppy, 1953), and even of a protein, insulin (Sanger and Thompson, 1953, Ryle, Sanger, Smith, and Kitai, 1955) Synthetic polypeptides have been produced (review by Elmore, 1961), and the attempt to understand how the activity of these big molecules varies with structure is probably the greatest challenge to the chemical pharmacologist at the moment and in the immediate future

Conclusion

Relationships between structure and ability to act at histamine receptors are more complex than those discussed in most other sections of this book The aminoethyl side-chain is important and this must be attached to a suitable unsaturated ring Most antihistamines appear to act by competition with histamine and to contain groups which could assist in binding the molecule to the histamine receptors In spite of the very high blocking activity of many of these compounds they are not effective in most asthmatic conditions, indicating that the symptoms are not due exclusively to histamine

XI

Conclusion

The Chemist's Contribution to Pharmacology

The *materia medica* of the pharmacopoeias of the mid nineteenth century came mostly from plants. The more obnoxious preparations of animal material of the eighteenth century (such as the *'Jus Viperinum'* or viper broth of the London Pharmacopoeia of 1745) had been deleted and work on synthetic drugs had not started. Today the *materia medica* is predominantly synthetic, with preparations from plants or animals in the minority. The chemist has a widely recognized part to play in providing these synthetic materials and in identifying the active constituents of plant extracts, such as alkaloids, or of animal material, such as hormones. Many of these latter may even be replaced by *synthetic material as the chemist develops better methods for synthesis*.

It is hoped, however, that this book will show that the chemist's contribution is not simply to provide material for others to test. He should be able also to provide information about the compounds he makes which may be directly relevant to their pharmacological properties. The structural formula of a compound will in itself provide information about the size and shape of the molecule and about the possible existence of isomers. If the electron distribution in the molecule is also taken into consideration, it should be possible to make a reasonable guess as to the acidic or basic character of the compound, its solubility, its stability, and the parts of it which might be involved in attachment to receptors.

Precise information about the size and shape of many molecules in the crystalline state has been obtained by X ray or electron diffraction measurements (for a summary, see Sutton, Jenkin, Mitchell, and Cross, 1958) and estimates of the size and shape of a new molecule can often be made with some degree of confidence. It is much more difficult to obtain quantitative estimates of electron distribution, however, as this cannot be measured directly, but must be inferred from other results. Nevertheless, a qualitative assessment can often be made and the predicted acidic or basic character, solubility or stability of a drug checked against what is found experimentally. The importance of solubility and stability as factors which influence pharmacological activity has long been realized, but it is only with the development of physical organic chemistry that it has appeared at all likely that it may be possible to relate these properties to chemical structure. The same is also true of the acidic or basic *character* of the drug. The relevance of the pK_a of an acidic or basic drug to its pharmacological activity has been

378

especially emphasized by Albert (1952, 1960) In many circumstances it can be used to calculate the proportion of the drug which is really active, and from the work of Brodie and others (see page 47, review by Brodie, 1956) it may also indicate the ease with which a drug may be absorbed or penetrate membranes The determination of the pK_a, moreover, may yield information about the electron distribution in the molecule The comparison of the pK_a values of a series of compounds, for example, may be used to assess the shift of electrons produced by various substituents

In fact, then, though a qualitative assessment of the electron distribution may be used to guess the physical properties of a new drug, it is usually the quantitative measurement of these physical properties which leads to more precise information about the electron distribution in the drug molecule This information will be particularly important in any attempt to assess how a drug may interact with a receptor, but it is, unfortunately, a subject about which the chemist has not very much information Developments in physical organic chemistry which lead to improved methods for estimating electron distribution will be very important indeed in pharmacology At the moment even the methods which could be used are not exploited fully, it is now a fairly common practice to measure the pK_a of a drug, but it is less often that information is sought from other (admittedly more specialized) sources, such as dipole moments or from infra-red or ultra violet absorption spectra

The chemist, however, has a further contribution to make, beyond providing the drugs and providing all possible information about their structure and physical properties he provides the ideas by which the actions of drugs are interpreted The whole theory of receptors, as discussed in Chapter I, is essentially a chemical one and it was this recognition of the relevance of chemical ideas in pharmacology, originally made by A J Clark, which made it possible to discuss the actions of drugs in scientific terms at all The recognition of the chemical principles underlying pharmacology may also (but does not always!) carry with it a striving after chemical standards of accuracy and of directness in experimental design

Actions of Drugs by Physicochemical Mechanisms

With the exception of *local anaesthetics* the drugs discussed in this book appear to act at receptors The problem has been to see how their activity varies with their size and shape and electron distribution, and to interpret these changes in activity in terms of ability to fit and activate a receptor The physical properties of the compounds have only appeared to be important in so far as they may affect access to the receptor sites

With the local anaesthetics, however, the absence of any correlation between activity and structure has been taken to indicate that activity depends upon physicochemical properties and various explanations for their action (none of which is particularly satisfactory) have been offered A similar situation is found with the actions of certain compounds on the central nervous system Many compounds of widely different structure will produce a general depression of the central nervous system, as opposed to a depression

of one particular part of it The effects observed are those of a general anaesthetic, loss of consciousness, and the blockage of reflexes, and the action has been described by the word 'narcosis' as well as by the terms 'anaesthesia' and 'general central depression'. As long ago as 1899, Meyer observed that the ability of a drug to produce narcosis appeared to be associated with its ability to dissolve in fat (Table XI 1) and suggested that it might be a consequence of the solution of the drug in the fatty tissues of the central nervous system, whose cells have a high fat content (see also, Baum, 1899, Overton, 1901, 1902)

TABLE XI 1
Narcosis of Tadpoles

	$\dfrac{C_{olive\ oil}}{C_{water}}$	Narcotic concentration (Mol /L.)	
Trional	4 46	0 0018	
Tetronal	4 04	0 0013	
Butylchloral	1 59	0 0020	
Sulphonal	1 11	0 0060	
Bromalhydrate	0 66	0 002	Decrease
Benzamide	0 6	0 002	in
Triacetin	0 3	0 010	potency
Diacetin	0 23	0 015	and
Chloralhydrate	0 22	0 020	solubility
Ethylurethane	0 14	0 040	
Monoacetin	0 06	0 050	
Methylurethane	0 04	0 040	
Ethanol	0 03	0 5	

$C_{olive\ oil}$ = concentration of drug in olive oil in equilibrium with a concentration, C_{water}, in the aqueous phase

Baum (1899)

Traube (1904) observed that there was a correlation between depressant activity and ability to lower the surface tension at an air–water interface, and suggested that the action of the compounds was a result of a lowering of the interfacial tension at the cell surface. It is unlikely that an air–water interface bears much resemblance to the cell surface and Warburg (1921) accordingly measured the adsorption of these compounds at a charcoal-water interface *He observed a correlation between activity and adsorbability* on this model and concluded that this supported Traube's hypothesis Other possible modes of action based on physicochemical properties have been suggested, for example, that the action depends upon an alteration of the permeability of the cell, possibly resulting in the reduction of the rate of removal of carbon dioxide (Höber, 1907, Winterstein, 1915, Lillie, 1909)

It is very difficult to decide which of these theories, if any, is correct In an homologous series of n aliphatic alcohols, for example, general central depressant activity in tadpoles increases in a geometrical progression with chain length, i e the activity rises in the ratio $1 \ n \ n^2 \ n^3$, etc (Meyer and Hemmi, 1935) If the logarithm of the concentration producing depression

is plotted against the chain length, a straight line is obtained until a maximum is reached with *n*-undecyl alcohol, above which activity drops sharply (Fig XI 1) A similar logarithmic increase in activity can be observed in other homologous series, for example with alkyl esters of *p* aminobenzoic acid (cf Table III 4) The concentrations of *n* aliphatic alcohols which reduce the surface tension of water to 63 dyne centimetres also decreases in a logarith-
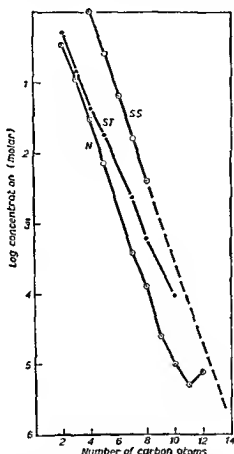
FIG XI 1   Properties of *n* aliphatic alcohols, ROH The number of carbon atoms in the chain (abscissa) is plotted against the logarithm of the concentration of alcohol 1, present in a saturated solution (curve SS) 2, producing narcosis of tadpoles (curve N) 3, lowering the surface tension of water to 63 dyne cms (curve ST) Results taken from Clark (1930), Meyer and Hemmi (1935), and Ferguson (1939)

mic fashion (Clark, 1930) but, then, so do all colligative properties, such as the lowering of vapour pressure, depression of freezing point, elevation of boiling point, osmotic effects, solubility, partition coefficients, and apparently even non specific adsorption at an enzyme or receptor surface (pages 211, 263) This is to be expected because in an homologous series the Gibbs Free Energy of the compounds is increased by a constant amount, roughly 2 kcal per mole, per methylene group and all these properties are dependent on the Gibbs Free Energy The results therefore, do not make it possible to decide which particular property may be responsible for the biological

activity of the compounds, though they indicate that narcotic potency is a physical property, like other colligative properties The decline in activity above a particular chain length is difficult to explain For some actions, such as the killing of certain bacteria (*Streptococcus Aureus*), it can be shown that the increase in activity with chain length is smaller than the decrease in solubility, consequently above *n* pentanol the bactericidal activity declines sharply because it becomes impossible to obtain enough *n* hexanol in solu

TABLE XI 2
Narcosis of Frogs and Mice

		Partition coefficient	Narcotic conc volume %	Conc. in oleyl alcohol corresponding to narcotic conc Mol /l
Frog	Nitrogen	0 05	9 000*	0 18
	Methane	0 54	760*	0 17
Mouse	Methane	0 54	370*	0 08
	Ethylene	1 3	80 0	0 04
	Nitrous oxide	1 4	100	0 06
	Acetylene	1 8	65 0	0 05
	Dimethyl ether	11 6	12 0	0 06
	Methyl chloride	14 0	6 5	0 07
	Ethylene oxide	31 0	5 8	0 07
	Ethyl chloride	40 5	5 0	0 08
	Diethyl ether	50 0	3 4	0 07
	tert Amyl alcohol	65 0	4 0	0 10
	Methylal	75 0	2 8	0 08
	Ethyl bromide	95 0	1 9	0 07
	Dimethyl acetal	100	1 9	0 06
	Diethyl formal	120	1 0	0 05
	CHCl=CHCl	130	0 95	0 05
	Carbon disulphide	160	1 1	0 07
	Chloroform	265	0 5	0 05

* Under pressure

Meyer and Hemmi (*1935*)

tion, even though the concentration required is theoretically, less than that of *n* pentanol which produced death of the bacteria (Ferguson 1939) This does not appear to be the explanation of the 'cut off' in narcotic activity at *n* undecanol, however (see Fig XI I) but it is possible that if the (thermodynamic) activities were plotted instead of the concentrations, the same phenomenon might be observed, i e the 'cut-off' may be due to the impossibility of obtaining a solution in which the (thermodynamic) activity of *n*-dodecanol and *n* tridecanol is high enough to produce the biological effect It is also possible that the inactivity of the compounds should instead be ascribed to their high surface activity resulting in their being adsorbed

extensively at all surfaces including, for example, the walls of the vessel in which the tadpoles are kept

Meyer and Hemmi (1935) measured the concentrations producing depression for a wide range of compounds and their partition coefficients between oleyl alcohol and water Although the concentrations varied several hundredfold, the equivalent concentration of the drug in oleyl alcohol varied less than 3-fold (Table XI 2) In similar experiments with an even wider variety of compounds, however, Meyer (1937) found that there was no correlation between depressant activity and surface activity at an air–water interface (Table XI 3) Although it is striking that there is so little variation in the

TABLE XI 3
Narcosis of Mice and Tadpoles

	$C_{narcotic,}$ mice	$C_{oleyl\ alcohol}$	Surface activity
Methane	370*	—	0
Nitrous oxide	100	0 06	0
Acetylene	65	—	0
Ethyl chloride	5	0 07	0
Ether	3 4	0 09	++
Methylal	2 8	0 08	++
Carbon disulphide	1 1	—	0
Carbon tetrachloride	0 6	0 07	0
Chloroform	0 5	0 07	0

	$C_{narcotic,}$ tadpoles	$C_{oleyl\ alcohol}$	Surface activity
Ethanol	0 33	0 033	+
n Propanol	0 11	0 038	++
n Butanol	0 03	0 02	+++
Valeramide	0 07	0 021	++
Phenazone	0 07	0 021	+
Ether	0 03	0 039	+
Amidopyrin	0 024	0 05	++
Benzamide	0 013	0 033	++
Salicylamide	0 0033	0 021	+
Phenobarbitone	0 008	0 048	+
o Nitroaniline	0 0025	0 035	+
Carbon disulphide	0 0005	0 03	0
Chloroform	0 00008	0 026	0
Thymol	0 000047	0 045	+

$C_{narcotic}$ mice = concentration (volume %) in air producing narcosis in mice
$C_{narcotic}$ tadpoles = concentration (Mol /l) in water producing narcosis in tadpoles
$C_{oleyl\ alcohol}$ = corresponding equilibrium concentration (Mol /l) in oleyl alcohol
0 = surface activity absent, + = surface activity weak, +++ = surface activity strong

* Under pressure

Meyer (1937)

concentration of drug in oleyl alcohol corresponding to the concentration producing depression, it is impossible to base satisfactory arguments about the mode of actions of drugs on experiments with model systems, such as oleyl alcohol and water, an air-water interface or a charcoal-water inter face Any attempt to reconstruct the situation in the living cell is bound to be unsatisfactory and lead to argument

It is possible, however, to demonstrate that these compounds are acting by a physicochemical mechanism without invoking the use of model systems Ferguson (1939) pointed out that in Meyer's experiments with volatile or gaseous compounds the conditions were approximately those of equilibrium, because the animal was maintained at a steady level of depression for some time Although the concentration of the drug in the biophase could not be measured, the chemical potential in all phases would be the same at equilibrium, and consequently the chemical potential in the biophase would be the same as that in the inhaled mixture, which could be measured Now the chemical potential

$$\mu = \left(\frac{\delta G}{\delta n}\right)_{T,P}$$

where G is the Gibbs Free Energy, n the number of molecules and T and P, the temperature and pressure, are constant If the chemical potential in a standard state is μ_0, the chemical potential, μ, of this substance present in the inhaled mixture with a partial pressure p_i (and behaving as a perfect gas) will be

$$\mu = \mu_0 + RT\ln(p_i)$$

The value $RT\ln(p_i/p_s)$, where p_i is the partial pressure in the anaesthetic mixture and p_s the saturation vapour pressure of the compound at the temperature of the experiments, should accordingly indicate the difference in Gibbs Free Energy between the molecule in the standard state and the molecule in the biophase, Ferguson therefore used the fraction p_i/p_s as an indication of the 'thermodynamic activity' of a compound He calculated this fraction, using the results of Meyer to obtain p_i and tables of physical constants for p_s, and found that although the compounds differed widely in the concentrations which produced depression (over 200-fold), the fraction p_i/p_s was relatively constant (Table XI 4) These results are very similar to those of Meyer and Hemmi (Tables XI 2 and 3), in which it was found that the concentration of the drug in oleyl alcohol corresponding to the concentration producing depression did not vary greatly from drug to drug The results of Ferguson's calculations, however, cannot be criticised on the grounds that they depend upon the validity of any particular model of the cell, their main assumption is that the experiments are performed in conditions of equilibrium They imply that the biological activity depends primarily upon the thermodynamic activity, i e that ΔG, the difference in Gibbs Free Energy between the standard and biologically active states, is roughly the same for

TABLE XI.4

Isonarcotic Concentrations of Gases and Vapours for Mice at 37° C.

Substance	Saturation pressure, 37° (p_s, mm)	Narcotic conc (% by volume)	Activity (p_t/p_s)
Nitrous oxide . . .	59,300	100	0·01
Acetylene . . .	51,700	65	0·01
Methyl ether . .	6,100	12	0·02
Methyl chloride . .	5,900	14	0·01
Ethylene oxide . .	1,900	5·8	0·02
Ethyl chloride . .	1,780	5·0	0·02
Diethyl ether . .	830	3·4	0·03
Methylal . . .	630	2·8	0·03
Ethyl bromide . .	725	1·9	0·02
Dimethyl acetal . .	288	1·9	0·05
Diethyl formal . .	110	1·0	0·07
Dichlorethylene . .	450	0·95	0·02
Carbon disulphide . .	560	1·1	0·02
Chloroform . . .	324	0·5	0·01

(p_t = narcotic concentration × 760 mm , for example, p_t/p_s for diethyl formal

$$= \frac{1·0 \times 760}{100 \times 110} = 0·07 \,)$$

Ferguson (1939).

most compounds, regardless of the absolute values of G. The situation can be expressed pictorially thus:
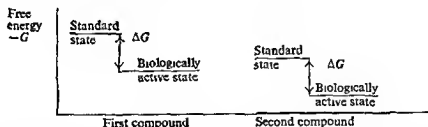
The fact that this fraction p_t/p_s is more or less constant was made use of in the development of the new general anaesthetic *Halothane* (see Suckling, 1957) Fluorinated hydrocarbons are manufactured for use in refrigerators, and it was thought possible that compounds of this type might be used as non-inflammable anaesthetics. From the value of p_s for the compounds, or even from their boiling-points, it was possible to predict the value of p_t and hence the concentration which would produce narcosis (Table XI 5).

Ferguson made similar calculations with other biological results, such as the ability of compounds to kill bacteria or insects and found that with these, too, the biological activity of many compounds appeared to depend upon their physical properties because the thermodynamic activity, p_t/p_s, was more or less constant (for a review, see Albert, 1960).

TABLE XL5

Boiling Point, Relationship Between Vapour Pressure, and Anaesthetic Concentration for Halothane and Related Compounds

	B P	p_s (20° C)	Anaesthetic conc (per cent)	p_i	p_i/p_s
		mm Hg		mm Hg	
$F_3C-CHBr_2$	73	104	0·4	3	0 03
F_3C-CH_2Cl	6	1,400	8 0	60	0 04
F_3C-CH_2Br	26	600	2 8	21	0 04
$F_3C-CH-I$	55	200	1 3	10	0 05
$F_3C-CHBrCl$ (*Halothane*)	50	243	0 9	7	0 03
$ClF_2C-CHCl_2$	72	110	0 8	6	0·05
$F_3C-CHBrCH_3$	49	260	2 2	17	0 07
F_3C-CCl_3	47	230	4 6	35	0 13

Suckling (1957)

The discussion so far has been concerned with volatile of gaseous compounds It is more difficult to come to any conclusion about the actions of drugs in solution because the chemical potential, μ, of the concentration, C, producing depression will be

$$\mu = \mu_0 + RT \ln (aC),$$

where a is the activity coefficient. In the gaseous phase the analogous correction factor (the fugacity) can reasonably be omitted, but for substances in solution, particular in body fluids, such as blood, or in physiological saline, the activities must be used rather than the concentrations, and there is considerable uncertainty about the values of these activity coefficients Brink and Posternak (1948), working with various kinds of nervous tissue, however, obtained results which appeared to be consistent with Ferguson's hypothesis, and it seems likely that on certain tissues the actions of many inert molecules (but not of all drugs), in solution as well as in the vapour phase, depends upon their thermodynamic activity and is unrelated to chemical structure

It is not clear, nevertheless, how this action is brought about. Mullins (1954) suggested that the interstices in the cell membrane become blocked with the molecules of inert drug and has been able to calculate, with some success, the thermodynamic activities of members of a homologous series which should produce the same effects Rang (1960) has endeavoured to test Mullins' hypothesis for the effects of n aliphatic alcohols on the mobility of *paramecia*, the depression of the response of the guinea pig intestine to acetylcholine, the depression of oxygen consumption by lung tissue, and depression of the release of hustamine from sensitized guinea pig lung His results do not agree particularly well with Mullins' hypothesis, but it is very difficult to come to any definite conclusion because of the uncertainty as to the validity of the assumptions made in calculating the activity coefficients

One conclusion, however, can be reached from this work and that is that the pharmacological actions of the 'unspecific' compounds are essentially a chemical problem

Some other drugs have also been discussed in this book, which are non-specific in a slightly different sense These are substances, usually of fairly large molecular weight, which have blocking activity at a number of sites Tubocurarine, for instance, has appreciable activity at ganglia as well as at the neuromuscular junction (page 134), more so than smaller neuromuscular blocking agents In particular, polymethylene *bis* triethylammonium salts, in contrast to polymethylene *bis* trimethylammonium salts, have comparable blocking activity at both these sites, and this activity increases almost in a logarithmic fashion up to a maximum at the heptadecamethylene compound It seems highly probable that the action of these compounds is due less to a high affinity for the acetylcholine receptor than to combination with receptor proteins by many Van der Waals' bonds This kind of binding may be particularly important with large molecules and it will be very interesting to see how far biologically active peptides and proteins will be found to be 'specific' in their actions As has already been mentioned (page 337), the mode of action of these large molecules is one of the great pharmacological problems of the immediate future, and it is a problem in which the chemist has a part to play which is not restricted to the elucidation of the structure of such compounds and to their eventual synthesis

Conclusion

Chemists have an important contribution to make in pharmacology, by synthesizing drugs, by providing information about them, and by assisting in interpreting how they act It is hoped that this book will enable them to see the importance of all these aspects of their work and lead to greater co-operation between chemists and all others engaged in pharmacological research

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APPENDIX

Note This is an extremely elementary account which is intended to provide the reader who knows no anatomy, physiology, or biochemistry with sufficient background to make the rest of the book intelligible

For general reading the reader is referred to *Introduction to Physiology*, by W H Newton (Arnold, London, 1948) and to the more detailed accounts, *Human Physiology*, by F R Winton and L E Bayliss (Churchill, London, 5th edition, 1962) and *Applied Physiology*, by Samson Wright (Oxford University Press, 10th edition, by C A Keele and E Neil, 1961) The chemist may find that *A Textbook of General Physiology*, by H Davson (Churchill, London, 2nd edition, 1960) gives a fascinating account of the scientific principles involved in certain aspects of physiology Reference may also be made to *The Principles of Human Physiology*, by E H Starling and C Lovatt Evans (Churchill, London, 13th edition, by H Davson and G Eggleton, 1962)

Part I: The Body as a Machine

Supply of energy – Extraction of food – Function of the liver storage of protein – Absorption and storage of fats – Combustion of fuel – Elimination of waste products – Maintenance of blood-cells – The lymph – Muscles structure and function – Involuntary and cardiac muscle, the heart.

Supply of Energy

In certain aspects the human body is a machine, converting chemical energy into mechanical and thermal energy In a petrol engine the energy is derived from the oxidation of hydrocarbons, but in the body it is obtained in three ways

    (1) By oxidation of carbohydrates,
    (2) By breakdown of proteins, and
    (3) By breakdown of fats

Normally all three are involved, but the first contributes by far the largest amount of energy

Extraction of Food

The petrol put into the tank of a car is pure fuel, the process of extracting it from crude oil having been performed at the refinery The body, on the other hand, is usually presented with its fuel in a crude state and has to extract what it can utilize From the time it enters the mouth to the time it reaches the colon before removal food is continuously subject to degradation and

extraction (Fig App I l) Carbohydrates, the chief source of energy, are first acted upon by the enzyme ptyalin which is secreted in saliva This begins the breakdown of polysaccharides, such as starch, into simpler ones, dextrin and maltose, but it has no action on fats or proteins The effect of
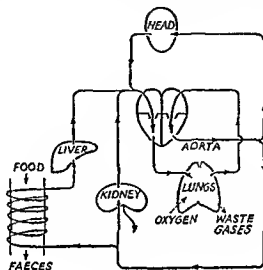
FIG. APP. I la
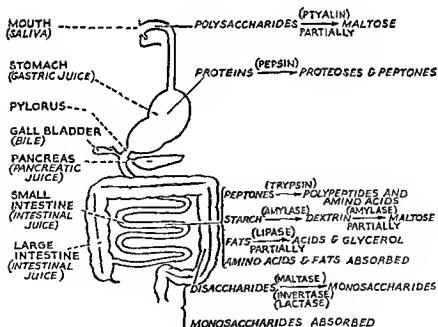
FIG APP I lb *The engine of the body*

ptyalin on food (or drugs) is not really important because the enzyme has only a short time in which to act The food passes rapidly into the stomach, where it is acted upon by the gastric juice This is acid and stops the action of ptyalin It contains the enzyme pepsin which degrades proteins to proteoses

and peptones It has little or no effect on fats and sugars, which are unaffected until the food passes out of the stomach, through the pyloric valve, into the intestine Here the food is acted upon by a secretion from the pancreas and by the bile

Pancreatic juice is alkaline and contains the enzymes trypsin (actually as an inactive precursor, trypsinogen, which is converted into trypsin in the intestine), amylase, and lipase, which act on proteins, carbohydrates, and fats respectively Trypsin resembles the pepsin of the stomach except that it acts much more rapidly, more powerfully, and in alkaline solution It completes the final breakdown of peptones to polypeptides and amino-acids The action of pancreatic amylase is similar to that of ptyalin, but, again, it is much more efficient and completes the conversion of starch into dextrin and thence into maltose Pancreatic lipase hydrolyses fats into fatty acids and glycerol

The bile flows into the intestine from the common bile duct This is supplied by the liver (through the hepatic duct) and by the gall bladder (through the cystic duct) It is in some extent a means of excreting waste products, such as the bile pigments which are formed from the destruction of old red blood cells It contains cholic acids, which have an emulsifying effect on fats and may thereby aid their absorption through the wall of the intestine

The alkaline juice secreted in the lower part of the intestine contains the enzymes maltase, invertase, and lactase, which affect the breakdown of disaccharides into monosaccharides

The intestine is normally continuously in movement like a concertina, and the intestinal wall contains vast numbers of fine capillaries filled with blood Amino acids and sugars are absorbed through the wall into these capillaries and so reach the blood stream The process of absorption does not appear to be just a passive diffusion There are definite mechanisms for the transport of these elemental food stuffs

Function of the Liver· Storage of Protein

It is at the intestinal capillaries that amino acids and sugars enter the body in a usable form The rich blood from these flows into the portal vein which supplies the liver, and in this organ glucose is converted rapidly into the polysaccharide glycogen and stored Glycogen can equally rapidly be reconverted to glucose, and so the body is able to maintain the glucose concentration in the blood at a fairly steady level whether it is at rest after a meal or working strenuously Muscle also has the property of storing glucose as glycogen, and can thus provide itself with a local store for its own needs Amino-acids are not stored by any such mechanisms but are slowly reconverted into proteins or broken down the nitrogen appearing as ammonia and urea

Fats do not enter the body through the intestinal capillaries but through other vessels in the intestinal wall called lymphatics It is thought that, to some extent at least, they enter these vessels in the form of fatty acids and

glycerol (having been converted into these in the intestine by pancreatic lipase) and are there reconverted into fat Fats are stored partly in the liver and are also laid down in adipose tissue under the skin

Combustion of Fuel

Fats and amino-acids are necessary for the replacement of vital constituents in the body such as lecithin and the body proteins The latter are re synthesized from the amino-acids absorbed, in consequence the proteins in the diet must contain suitable ('essential') amino acids In the same way, certain other substances (Vitamins) must be present in the diet, their absence deprives the body of essential substances which it cannot make itself Proteins and fats have an additional function The breakdown of proteins furnishes an appreciable amount of energy, but the oxidation of fats produces more, even, than that of a corresponding amount of carbohydrate The combustion of 1 g of either protein or carbohydrate provides 4 kcals , combustion of 1 g of fat provides 9 kcals All three types of fuel yield energy by enzymic oxidation or degradation The blood-stream not only provides access to oxygen but also ensures the distribution of the fuel The blood consists of cells suspended in a straw-coloured fluid called plasma The latter is an aqueous mixture which contains the ions sodium, potassium, calcium, chloride, bicarbonate, phosphate, sulphate, and lactate as well as the solutes glucose and urea, and cholesterol, fats, lecithin, and protein It is isotonic with 0 93 per cent sodium chloride and the buffering system makes its pH about 7 4 The red blood cells contain haemoglobin, and it is they which carry oxygen to all parts of the body and which are also partly concerned with the elimination of the principal waste product, carbon dioxide The haemoglobin is converted into oxyhaemoglobin during the passage of the blood through the capillaries of the lungs Red cells containing oxyhaemoglobin are carried in the arteries to the capillaries of organs and muscle where oxidation of fuel occurs Here the oxyhaemoglobin is converted to reduced haemoglobin and carbon dioxide is taken up by the blood The carbon dioxide is carried to the lungs in the venous blood – which is darker than arterial blood – chiefly as the bicarbonate anion, HCO_3^-, present in both red cells and plasma In the lungs the bicarbonate anion is reconverted into carbon dioxide and exhaled together with water vapour Other waste products are removed from the blood stream by the kidneys

Elimination of Waste Products

The action of the kidney is a three-stage process The kidney contains millions of nephrons, units which consist of a capsule joined to a convoluted tubule (Fig App I 2) The first process is a simple filtration, molecules which are small enough pass through the walls of the capillaries in the capsule They then pass down the tubule and may be reabsorbed through the walls of the tubule and so returned to the blood stream It is only substances which fail to be reabsorbed which are excreted into the ureter and so into the bladder

The process of reabsorption, like absorption through the walls of the intestine, involves a definite transporting mechanism
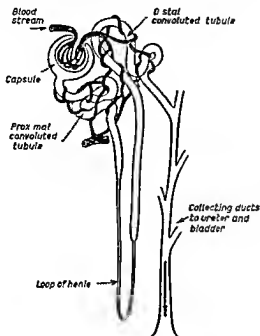
FIG APP 1 2 *Filtration at the kidney*

Maintenance of Blood-cells

In its turn, the blood is kept supplied with fresh cells, both red and white, by the bone-marrow and the spleen The latter is n small organ, situated to the left of the stomach Old cells are broken down into bile pigments and leave the body via the bile in the faeces
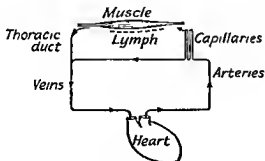
FIG APP 1 3 *Lymph flow*

The Lymph

The lymph is a transudate of plasma, that is, it consists of those substances water, salts, and a very little protein, which can pass through the walls of ordinary capillaries It provides a bath in which the muscles may work The

importance of the lymph in connexion with fat absorption has already been mentioned The fluid is collected away into thin walled ducts and returned by the principal of these, the left and right thoracic ducts, into the subclavian and internal jugular veins (Fig App I 3)

Muscles: Structure and Function

The parts of the body which actually perform mechanical work are the muscles These are of three types called

1. Plain or involuntary
2. Striated, striped, skeletal, or voluntary
3. Cardiac

The first is found in all those parts of the body which function automatically (e g the intestines), the second in those controlled by the will (e g the limbs) Cardiac muscle, which is also striated, falls into a class by itself All three are composed of individual fibres Plain muscle fibres are long, oval, nucleated cells surrounded by a thin membrane These are embedded in a common connective tissue and cross linked by fine filaments Voluntary muscle fibres are longer than plain muscle fibres, they are often up to 5 cm long and about 100μ in diameter They consist of contractile substances and a sheath which encloses it called the sarcolemma The nuclei without which no cell can live, are usually found just below the sarcolemma The contractile substance is made up of long threads called myofibrils surrounded by a fluid called the sarcoplasm It appears to consist of alternate dark and light transverse bands called sarcomeres, about 2–3 μ apart The bands are not discontinuities in the structure of the cell They are caused by differences in the optical activity of the cell fluid (i e they are anisotropic layers) When a fibre is stimulated (e g by an electric shock), a wave of contraction passes along it the sarcomeres draw closer together, and it bulges (Fig App I 4) As the wave passes on, the sarcomeres separate once more and the fibre returns to normal The wave of contraction is accompanied by a flow of electric charge along the fibre in the same direction

FIG APP I 4 *Wave of muscle contraction (diagrammatic)*

(After Sharpey Schafer)

Muscle fibres either respond fully to a stimulus or not at all When a muscle is stimulated there is a latent period of about 2 msec before it begins to contract The effect reaches a maximum in about 40 msec and finally disappears after about 200 msec If the muscle is stimulated again before the effect of the first stimulus has passed off, nothing may happen if the interval between the stimuli is less than the absolute refractory period of the muscle, which is about 5 msec If it is a little longer, the muscle responds only once to the two stimuli, but the contraction is greater than that produced by one stimulus, although it is nothing like twice the size When the stimuli are

further apart, about 200 msec , two responses are observed The second is modified by the first, and the two become identical only if the stimuli are further apart still Repeated stimuli very close together cause a tetanus, a prolonged contraction of the muscle (Fig App I 5)
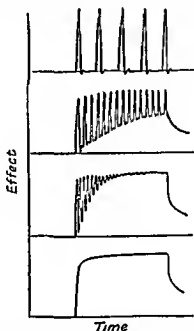
FIG APP I 5 *Effect of increasing rate of stimulation on response of a nerve-muscle preparation The responses gradually fused and in the bottom tracing a tetanus has been set up*

It seems likely that the contraction of muscle is caused by a change in the packing of the myosin molecules (of which the contractile substance is thought to be made) from the β to the α structure It is known from X ray evidence that the α and β forms of keratin, the protein constituent of hair and
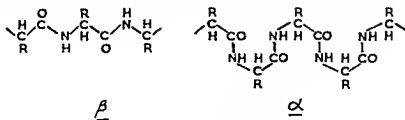
FIG APP I 6

wool, differ only in this way (Fig App I 6) The shrinking of wool is caused by a change from the β form to the α

The chemical reactions involved in the contraction of muscle must supply energy, but this is actually needed not for the contraction, but for the relaxation afterwards The contraction is an exothermic process The energy

for the reconversion of the myosin of voluntary muscle into the stretched
form is obtained from the enzymic breakdown, by myosin itself, of adeno-
sine triphosphate (ATP) to adenosine diphosphate (ADP) ADP is at once
re-phosphorylated by creatine phosphate (CP), and the energy for the re
phosphorylation is obtained by the breakdown of muscle glycogen to lactic
acid which accompanies the re phosphorylation of creatine The latter is a
much slower process than the others, and in consequence repeated contrac
tion of the muscle results in the exhaustion of supplies of CP and the ac
cumulation of lactic acid There is a limit to the amount of lactic acid which
muscles will tolerate and they become fatigued unless the lactic acid is
removed The mechanisms which exist for this depend on an efficient circu-
lation of the blood If oxygen is available, lactic acid can be oxidized to
pyruvic acid, but it can also be resynthesized into glycogen and thus replace
losses incurred in the re phosphorylation of creatine This occurs in the liver
as well as in the muscles, and the necessary energy is obtained from the
oxidation of food stuffs (including, as just mentioned, that of lactic acid
itself) The whole process can thus be represented

$$
\begin{array}{c}
\text{ADP} \dashrightarrow \text{CP} \dashrightarrow \text{Lactic acid} \dashrightarrow \text{Fuel including} \\
\boxed{\textit{VERY FAST}} \quad \boxed{\textit{SECONDS}} \quad +\text{O}_2 \quad \textit{MINUTES} \\
\text{ATP} \dashrightarrow \text{C} \dashrightarrow \begin{array}{c}\text{Muscle}\\ \text{glycogen}\end{array} \dashrightarrow \text{Pyruvic acid etc} \\
\text{In liver} \\
\text{(Some liver glycogen)}
\end{array}
$$
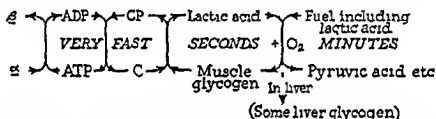
When the circulation ceases at death, the last process will stop when supplies
of oxygen are exhausted It is the accumulation of lactic acid consequent
upon this which produces the state of general stiffening of the muscles which
is known as *rigor mortis*

About involuntary and cardiac muscle less is known Both resemble
voluntary muscle in their general behaviour (e g refractory period, etc) but
whereas the latter is stimulated by nervous impulses, both the former types
contract spontaneously, although they may be influenced by external con
ditions and nervous stimulation In the heart, rhythmic contraction is
initiated by some mechanism which is not understood at a point called the
pace-maker The biochemical processes involved in the contraction of in
voluntary and cardiac muscle may be much the same as those in voluntary
muscle Because it is vital that the heart should not become fatigued, it is
provided with a special blood supply of its own called the coronary system
It is important to remember this when considering the action of drugs on the
heart

Part II: Nervous Controlling Mechanisms

Function of nerve-cells – Sensory and motor fibres – The brain, a coordinating centre – The spinal cord – Voluntary and involuntary nervous systems – Sympathetic and parasympathetic – Structure of nerve-cells, ganglia – Nerve fibres – Peripheral nerve-endings – Nervous connexions of the spinal cord and periphery – Relations between the structure of nerves and their function – Nervous conduction – Nervous connexions in the brain

Function of Nerve-cells

The previous section has described very briefly the workings of what may be called the engine of the body. The body, however, is more than an engine, it is capable of detecting changes affecting it and readjusting itself to them

The two sets of apparatus, for detection and for reaction, are remarkably similar Both involve special cells called nerve-cells These consist of a nucleus attached to a long fibre which can conduct impulses (the various types are discussed on page 444) The fibre links the nerve-endings near the surface of the body or in its important organs with a junction with some other nerve fibre The individual fibres are collected together like the strands of an electric cable, which is the nerve proper or nerve 'trunk' (Fig App II 1)

FIG APP II 1 *Cross section of a giant nerve trunk The individual nerve fibres may be seen collected together into six separate nerves*

Sensory and Motor Fibres

There are two kinds of nerve Both can transmit impulses in the form of electric charges, but whereas one type carries impulses *away* from the surface or organ, the other type carries impulses *towards* the surface or organ. The former are called afferent or sensory, the latter efferent or motor Sensory nerves are stimulated when some change occurs at the environment of the peripheral nerve endings This may be a change of temperature, an electric shock, or a mechanical disturbance, such as a pin prick Motor nerves function in the opposite way, an electrical impulse travelling to the nerve-endings produces a mechanical response in the form of a muscle twitch or a change in the activity of an organ

In very low orders of life, a sensory nerve is connected directly with its

appropriate motor nerve at what is called a synapse (Fig App II 2) When the part of the animal near the sensory nerve is disturbed, the impulse travels to the synapse, where, if it is strong enough, it stimulates the motor nerve This causes a counter action to be taken by the muscles near the motor nerve endings As these nerve endings are usually very near to the sensory nerve endings, the part of the animal disturbed and the part which reacts is effectively the same

FIG APP II 2 A = *Sensory (Afferent) nerve endings* E = *Motor (Efferent) nerve endings*

The Brain, a Co-ordinating Centre

In higher forms of life the impulses which the body receives from the enormous numbers of sensory nerves have to be co ordinated The information 'my right leg has been stung just below the knee' elicits not only a twitch from the right leg but also movement of an arm to swat the offending insect or rub the injured part This demands something more like a telephone exchange than an automatic relay, which is what the synapse really is Even this analogy is inadequate because the telephone exchange must be intelligent The actual mechanism, which is called the central nervous system (CNS), is better compared with an army staff in continuous communication with its inferiors Anatomically it consists of the brain and spinal cord (Fig App
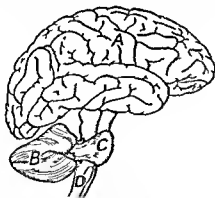
FIG APP II 3 A = *Cerebrum* B = *Cerebellum* C = *Pons* D = *Medulla oblongata*
(*After Schwalbe*)

II 3) The commander in chief and his immediate advisers can be considered to be located at the cerebrum (A) His juniors are to be found in descending order of seniority, lower down (at B, C, and D) These lower 'centres' of the brain, as they are called, are responsible for functions which are not normally disturbed by the higher centres The respiratory centre, for instance, is in

the medulla (D) and functions automatically unless we exercise our will to interfere This voluntary action would involve the higher centres of the cerebrum which 'monitor' the control of respiration usually exerted by the centre in the medulla This kind of monitoring is important and is especially common in still lower parts of the CNS (i e in the spinal cord)

The Spinal Cord

This lies inside the hollow part of the vertebrae which form the spine (Fig App II 4) It is continuous with the brain, and the boundary between the brain and spinal cord is purely arbitrary Both are covered by the same

(Back)

SPINAL CORD

SPINAL CANAL AND CEREBRO-SPINAL FLUID

(Front)

(Back)

ARACHNOID MATER
DURA MATER
PIA MATER

GREY MATTER

WHITE MATTER

(Front)
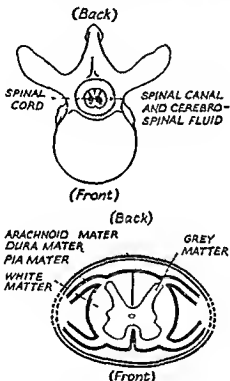
FIG APP II.4 *Coverings of spinal cord*

membrane and surrounded by the same fluid, the cerebrospinal fluid (CSF) The substance of the cord consists of white matter with an inner core of grey matter The white matter is made up of nerve-fibres each of which carries its own messages to or from the brain Sensory and motor nerves supplying the various parts of the body join the cord at points along its length corresponding to most of the spinal vertebrae Sensory nerves join the posterior parts of the cord where all sensory fibres are collected together, motor fibres join the anterior parts

The grey matter consists of nerve cells with only very short fibres which are arranged so as to supply a connexion between a sensory nerve and its ap-

propriate motor nerve (Fig App II 5) The whole arrangement is called a reflex arc It permits either of reflex (i e automatic) control via the synapses of the grey matter, or of direct control by the brain through the connexions in the white matter When a person is pricked in the arm, the twitch which his arm gives is a result of stimulation via the synapses of the grey matter If the person, however, is expecting a hypodermic injection, his brain will monitor the impulses and he may keep his arm still
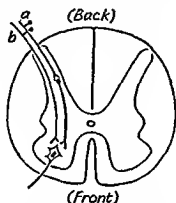
FIG APP II.5 *Reflex arcs*
There are usually two junctions in the grey matter (a), but sometimes only one (b)

Voluntary and Involuntary Nervous Systems

Nerves have so far been divided into two classes, sensory and motor Whereas the former serve merely to carry information to the CNS the latter have the more complex task of controlling the various parts of the body They are of two types, autonomic ('vegetative' or 'involuntary') and voluntary Autonomic nerves primarily control the organs of the body, the heart, stomach, liver, etc , which carry out the mechanical acts of life Voluntary nerves control the limbs, etc , which are directly operated by the will

Sympathetic and Parasympathetic

Most hollow organs (or viscera) of the body are doubly innervated They can be either stimulated or depressed, one set of nerves being responsible for stimulation and another set for depression This leads to a further division of autonomic nerves into sympathetic and parasympathetic The pupil of the eye (iris), for instance, is constricted by parasympathetic stimulation whereas sympathetic stimulation dilates it The divisions may be summarized as follows
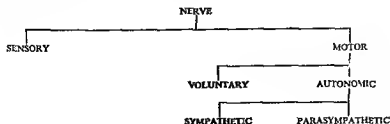
Table App II I shows the effects of sympathetic and parasympathetic stimulation on various organs The effects are easily summarized

TABLE APP II 1

The Effects of Stimulation of the Sympathetic and the Parasympathetic Nerves on the Chief Organs of the Body

Organ	Sympathetic	Parasympathetic
Blood vessels	Constriction, except coronary vessels which are dilated	Nil (except in certain special cases where dilatation occurs, and in the coronary vessels which are constricted)
Heart	Acceleration and augmentation	Inhibition
Eye		
Iris	Contraction of Radial Muscle (Mydriasis)	Contraction of Circular Muscle (Miosis)
Ciliary muscle	Nil	Contraction
Skin		
Sweat secretion	Augmentation	Nil
Erection of hairs	Increased	Nil
Salivary glands	Slight viscid secretion	Free secretion and vaso-dilatation
Stomach		
Contractions	Inhibition	Augmentation
Secretions	—	Increase
Sphincters	Contraction or relaxation	Relaxation or contraction
Intestinal movements	Inhibition	Augmentation
Gall bladder	Relaxation	Contraction
Liver	Glycogenolysis	Nil
Spleen	Contraction	Nil
Pancreatic secretion	—	Increase
Bronchial muscles	Relaxation	Contraction
Bronchial secretion	Nil	Increase
Suprarenal glands	Secretion	Nil
Ureter	Relaxation	Contraction
Bladder		
Fundus	Relaxation	Contraction
Sphincter	Contraction	Relaxation
Uterus	Contraction and relaxation	

(Wilson and Schild, 1952, *Clark s Applied Pharmacology*, J and A Churchill, reproduced by permission.)

Sympathetic stimulation may be said to prepare an animal for fight or flight, parasympathetic stimulation for post prandial relaxation The former favours instant violent action, the body is furnished with extra fuel (glucose) wider air passages (by dilatation of the bronchi), and a higher heart rate and blood pressure to ensure a quicker turn round of fuel and waste gases In the latter, conditions are made favourable for digestion, for storage of fuel against future needs, for rest, and for repair of wear and tear

Structure of Nerve-cells; Ganglia

Although the fibres of nerve cells are much bigger than the nucleus and possess the property of conducting impulses, the existence of a nucleus is essential for the life of the whole nerve cell There are three ways in which the nucleus and the fibres are found to be arranged
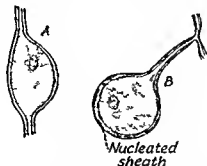
FIG APP II 6 A = Bipolar B = Unipolar

The first, the bipolar type (Fig App II 6), is the simplest possible, the nucleus is a bulge at a point on one side of the fibre This type, however, is found only in the lower orders of life, and it is a more developed form the unipolar type, which is found in man In this, only one fibre is directly attached to the portion of the cell which contains the nucleus, it splits into two a little distance away The cell is protected by a nucleated sheath This
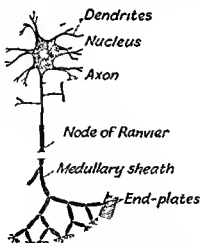
FIG APP II 7 *Multipolar nerve cell*

kind of cell is rather like a relay station, and occurs particularly frequently in sensory nerves just before these join the spinal cord In the same way as individual fibres are collected together in a nerve trunk, so these nuclei are collected together in what is called a ganglion

The third type of cell is called multipolar and is very common in the motor nervous system (Fig App II 7) It has many 'processes' called dendrites, but only one fibre, the axon, carries impulses away to the roots

These cells are found in the spinal cord and in the relay stations (ganglia), if any, between the cord and the peripheral nerve-endings The roots of the previous fibre arborize round the cell, and the ganglion is therefore different in structure from that formed of unipolar cells

Nerve-fibres

The nerve-fibre itself is surrounded by an elastic nucleated membrane called the primitive sheath or neurilemma (Fig App II 8) There may be, in addi-

Node of ranvier
Nucleated sheath
Sheath cell nucleus
Nerve fibrils
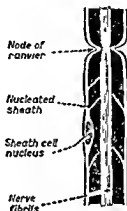
FIG APP II 8 *Nerve fibre (longitudinal section)*

tion, a medullary sheath of myelin (protein) between the neurilemma and the axon Nerve-fibres are accordingly classified as medullated and non-medullated (or myelinated and non myelinated)

Peripheral Nerve-Endings

The point where the nerve joins muscles or organs is called the nerve ending The term neuromuscular junction is usually restricted to the junction of a voluntary motor nerve with skeletal muscle At the nerve ending motor nerve-

End plate regions on muscle cell
Medullary sheath
Two muscle cells

FIG APP II 9

Nerve trunks
Vessel
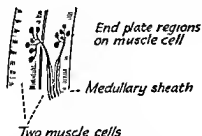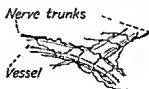
FIG APP II.10 *Nerve plexus.*

fibres branch two or three times, and each branch goes to a muscle-fibre (Fig App II 9) The neurilemma becomes continuous with the sarcolemma and the medullary sheath stops short Nerves supplying involuntary muscle may finish in plexuses (i e networks of fibres which contain some free endings) (Fig App II 10) Sensory nerve-endings are of various types Some resemble

motor nerve-endings, as they are junctions with muscle-cells Some form networks like the involuntary plexuses (around hairs, for example), and in special touch corpuscles and end-bulbs the fibre originates from a sort of capsule (Fig App II 12 and 13)
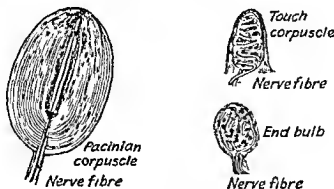
FIG APP II 11 *Sensory nerve-endings*

Nervous Connexions of the Spinal Cord and Periphery (Fig. App. II. 12 and 13)

As has already been implied, the distribution of the various types of nervous apparatus in the body is related to the function for which it is required Sensory fibres, for instance, are usually medullated They run direct from the periphery to the posterior roots of the cord via the spinal ganglia, where the nuclei of these unipolar cells are collected together Many of these ganglia are arranged in pairs, one each side of the cord, corresponding with the segments of the vertebral column
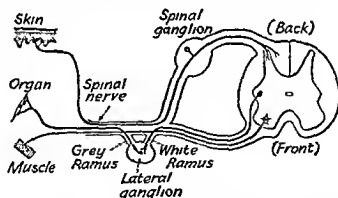
FIG APP II 12 *Nervous connexions of the spinal cord and periphery*

Voluntary fibres are likewise medullated, and run direct from their dendrites and nuclei in the anterior part of the cord to their peripheral terminations They are multipolar cells It will be appreciated that both sensory and voluntary fibres are very long

The connexions of the autonomic nervous system proceed through ganglia of the multipolar-cell type Those of the sympathetic system are located near the spinal cord and arranged very like the sensory spinal ganglia, one each side of approximately each vertebra of the spine Those corresponding

P

to the upper four thoracic segments are combined in the large stellate ganglion, and those to the upper four cervical in the superior cervical ganglion These sympathetic ganglia constitute what is called the sympathetic chain They are connected with the spinal cord and vertically with one another Medullated fibres from the multipolar cells of the intermedio-lateral tract combine with voluntary fibres to form the spinal nerve They then break away and join the ganglia, where they arborize around similar multipolar cells Non
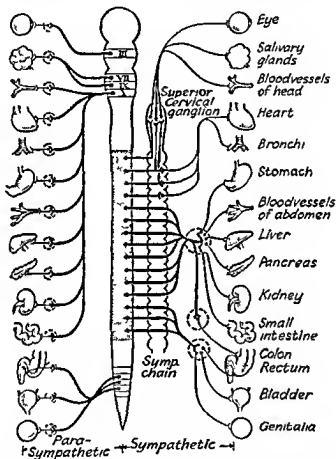
FIG APP 11 13 *Autonomic nervous system (after Meyer and Gottlieb)*
Note – *Only one side of cord is shown Preganglionic fibres shown as thick lines*

medullated fibres from these cells rejoin the spinal nerve The preganglionic fibres are called the white rami and the postganglionic fibres the grey rami from their appearance The fibres of the white ramus are medullated but very fine, they are only 2-3 μ in diameter, whereas motor fibres are 15-20 μ Those of the grey ramus are usually non medullated and long, they run direct from the ganglion to the tissues Most of the fibres of the white ramus terminate in the lateral ganglia, where one preganglionic fibre may serve to relay

impulses to as many as twenty or more postganglionic fibres, but not all do this Some link one part of the cord with ganglia several positions up or down the chain instead of with the nearest one Some even pass right through a lateral ganglion without having a synapse in it In this case the fibres terminate either in a solar ganglion such as the coeliac ganglion or in a terminal ganglion near the periphery or organ (Fig App II 14) Whatever happens, the nerve appears to have only one synapse between cord and termination Normally, therefore, the connexions of the sympathetic nervous system are

(1) Short, medullated, preganglionic fibres to the sympathetic chain, and
(2) Long, non medullated, postganglionic fibres, with the exceptions just mentioned
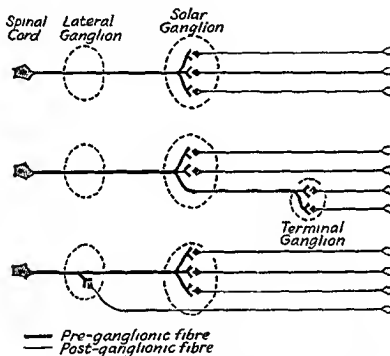
FIG APP II 14 *Alternative arrangement of pre and postganglionic fibres in splanchnic and inferior splanchnic nerves (after Langley)*

Nervous connexions of the parasympathetic system all pass through ganglia, but, unlike those of the sympathetic system, these are usually situated near the tissues they innervate instead of near the spinal cord In consequence, preganglionic parasympathetic fibres are long and postganglionic fibres, which are usually non medullated, are short Parasympathetic fibres leave the cord at four points (Fig App II 13)

1 From the mid brain via the third nerve to the ciliary ganglion From there short ciliary nerve fibres run to the muscles of the eye
2 From the medulla (a) via the seventh and ninth nerves to ganglia and thence to the nose and mouth

(b) via the tenth (Vagus) and eleventh nerves to ganglia either on the nerve or in the walls of the oesophagus, stomach, small intestine, etc

3 Certain fine medullated fibres leave the posterior spinal roots (in spite of these being afferent nerves) and are responsible for the dilatation of some peripheral blood vessels

4 The white rami of the second, third, and fourth sacral nerves pass through the corresponding lateral ganglia without having a synapse and form the pelvic nerves They terminate in the ganglia of the pelvic plexus, from whence fibres are distributed to the colon, etc

It should now be possible to understand Fig App II 13, which summarizes the controlling mechanism The double innervation of the vital organs will be seen The heart, for instance, is supplied with parasympathetic fibres by the vagus nerve and with sympathetic fibres from the ganglia of the sympathetic chain A further sympathetic connexion exists in the suprarenal gland Stimulation of this gland results in the discharge into the blood stream of a mixture of chemicals which stimulates all or nearly all, the sympathetically innervated organs to which it is conveyed Stimulation of the splanchnic nerve which innervates the suprarenal gland can thus prepare an animal for fight or flight in a matter of about a second (the time taken by the blood stream to convey the chemicals to the various organs) It may be regarded as the equivalent of dialling 999 on the telephone It will be seen that spinal reflex arcs involve sensory voluntary and sensory sympathetic connexions rather than sensory parasympathetic ones

Relations Between the Structure of Nerves and their Function

It is now possible to summarize in a general way the relations between the structure of nerves and their function

Sensory—Long medullated
Types of ending capsule plexus or end plate

Motor—Long medullated 15–20 µ in diameter
Types of ending end plate

Sympathetic—Preganglionic short, thin medullated 2–4 µ in diameter
Postganglionic long, non medullated
Types of ending plexus

Parasympathetic—Preganglionic long medullated
Postganglionic short, non medullated
Types of ending plexus.

Spinal Cord
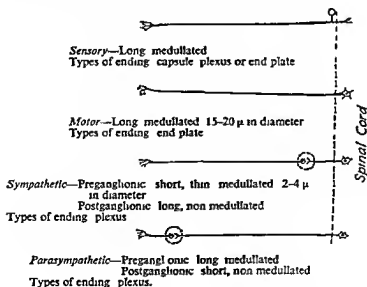
Nervous Conduction

Nerve fibres resemble muscle fibres in their response to stimulation, they either conduct fully or not at all An anaesthetic drug, for instance, does not lower the efficiency of conduction of individual fibres, but reduces the proportion of fibres which conduct There is an absolute refractory period of 0 4–0 5 msec before the nerve can be induced to respond again After this it is hyposensitive and requires a bigger stimulus than normal It is only after a relative refractory period of 3–5 msec that the nerve returns completely to normal If the nerve is stimulated very rapidly, the response evoked will drop when the interval between impulses is less than the relative refractory period If the interval is as small as the absolute refractory period, the response may suddenly be reduced to half because only one impulse in two is effective Oxygen is vital for the efficient performance of nerve but, if supplies are adequate, an isolated nerve preparation can conduct impulses for many hours
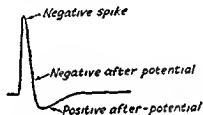
FIG APP II 15 *Action potential of nerve fibre*

The electrical changes which take place during nervous conduction can be amplified and detected on the screen of a cathode-ray tube Between two electrodes, one inside and the other outside a nerve fibre, there is a resting potential difference of 60–80 mv, the outside is positive with respect to the inside When the nerve is stimulated, a short negative spike is seen on the screen, followed by negative and positive after-potentials of very much smaller magnitude and longer duration (Fig App II 15) If both electrodes are on the nerve surface, the potential difference changes sign as the impulse travels along the nerve It has been shown that there are actually three types of mammalian nerve-fibres, *A*, *B*, and *C*, which have the properties listed in Table App II 2

Fibres of class A provide most of the afferent and efferent connexions of voluntary muscle Those of class B are found only in the autonomic nervous system and form the bulk of preganglionic nerves A few are found in post-ganglionic nerves, but these consist mainly of class C fibres, which are also found in the dorsal (sensory) spinal roots It is interesting to note that whereas lack of oxygen affects the fibres in the order B, A, C, the drug cocaine blocks class C fibres first and class A last The rate of conduction also seems to be related to the thickness of the fibre, and the greater sensitivity of thin fibres to cocaine may be associated with their relatively greater surface area.

Unlike the waves on contraction in muscle, nerve-impulses do not demand excessive amounts of energy, although it appears that the reactions involved in supplying it are probably similar to those which occur in muscle.

TABLE APP. II 2
Some Properties of Three Groups of Mammalian Nerve-fibres after Grundfest

Group	A	B	C
Diameters of fibres, microns	20 to 1	3	Unmyelinated
Conduction velocity, metres per second	100 to 5	14 to 3	2
Spike duration, milli-seconds	0 4 to 0 5	1 2	2 0
Negative after potential			
Size, per cent., of spike	3 to 5	None	3 to 5
Duration milli-seconds	12 to 20		50 to 80
Positive after potential			
Size, per cent., of spike	0 2	1 5 to 4	1 5
Duration, milli-seconds	40 to 60	100 to 300	300 to 1,000
Absolute refractory period, milli-seconds	{a 0·4 to 1·0} {8 0·6 to 1·0}	1 2	2 0

(Winton and Bayliss, 1948, *Human Physiology*, J and A Churchill reproduced by permission)

Nervous Connexions in the Brain

These are far more complicated than those in the periphery Many parts of the brain appear to contain 'centres' which are concerned with particular activities The greater part of the cerebrum is associated with voluntary movement and specialized functions, such as speech and sight. Autonomic centres are located lower down

Many therapeutically important drugs produce their effects by actions on the central nervous system, for example, general central depressant drugs, analgesic drugs, anticonvulsant drugs, antitussive drugs, anti-emetic drugs, antipyretic drugs, analeptic drugs, and drugs which are effective in mental illnesses With the exception of general central depressant drugs (briefly considered in Chapter XI) and drugs acting on the spinal cord, there is much uncertainty about the precise site of action of these drugs and great difficulty in assessing whether the effect observed is that of the drug itself, or of some metabolite produced from it Even though surgical techniques make it possible to administer drugs directly into cavities in the central nervous system (such as the cerebral ventricles), penetration to sites within the central nervous system is still uncertain It would, for instance, be limited if the substance constricted the blood-vessels present in the tissues Because of this, the discussion of the actions of drugs in chemical terms is limited in this book to actions outside the central nervous system, such as those at sites in the peripheral nervous system and directly on smooth muscle

Index

Numbers in italics indicate the page on which the structural formula of the compound may be found.